Factors Influencing Skeletal Muscle Na^+, K^+-ATPase and Plasma $[K^+]$ During Exercise in Humans

Submitted by

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Abstract

The sodium-potassium adenosine triphosphatase enzyme (Na^+,K^+-ATPase) regulates muscle cell [Na^+], [K^+] and muscle membrane potential, therefore playing a fundamental role in muscle excitability. This dissertation primarily examined the effects of acute exercise, training status and chronic inactivity on Na^+, K^+-ATPase content and activity in human skeletal muscle. Since Na^+, K^+-ATPase activity also regulates plasma [K^+] at rest and during exercise, a secondary focus of this thesis was to examine relationships between muscle Na^+,K^+-ATPase and plasma [K^+] responses during and following exercise.

Study 1. Measurement of Na^+, K^+-ATPase activity in human skeletal muscle is difficult due to low inherent activity and high unspecific ATPase activity. Therefore the first study involved modification of the K^+-stimulated 3-*O*-methylfluorescein phosphatase (3-*O*- MFPase) assay for measurement in human skeletal muscle. Factors investigated included enzyme kinetics, sample treatment and ligand concentration. The specificity of the assay for Na^+,K^+-ATPase was confirmed by inhibition of the maximal K^+-stimulated 3-*O*- MFPase activity by ouabain. 3-*O*-MFPase activity was maximal using a multiple freeze-thaw treatment of the homogenate, a 10 mM KCl activating concentration and a 3-*O*- MFP substrate concentration of 160 μM. The maximal K^+-stimulated 3-*O*-MFPase activity in quadriceps muscle homogenates in seven healthy untrained subjects was (mean±SE) 292±10 nmol min^{-1} g^{-1} wet wt (1745±84 pmol min^{-1} mg^{-1} protein). The intra-assay variability was 8.1% and the inter-assay variability was 5.3%. These modifications optimised the 3-*O*-MFPase assay allowing valid, reliable measurements of Na^+,K^+-ATPase activity in small samples of human skeletal muscle.

Study 2. The acute effects of fatiguing exercise on Na^+, K^+-ATPase activity has not previously been investigated, therefore the second study examined the effects of

fatiguing knee extensor exercise on muscle 3-*O*-MFPase activity and plasma [K^+]. Eight untrained men undertook an incremental exercise test on a cycle ergometer to determine $\dot{V}O_2$ peak and a separate muscle fatigue test (MFT) comprising 50 maximal voluntary contractions of the knee extensors. A muscle biopsy was taken from the vastus lateralis at rest and at fatigue in the MFT to determine maximal *in-vitro* K^+-stimulated 3-*O*-MFPase activity, and muscle metabolites. Muscle Na^+, K^+-ATPase content (^3H ouabain binding site content) and fibre types were also measured in resting muscle. Arterialised-venous blood was sampled from a dorsal hand vein, before, during and after both the MFT and $\dot{V}O_2$ peak test, and was analysed for plasma [K^+]. In the MFT, peak torque declined by 49.5±2.8 % (P<0.05), muscle ATP, PCr, and glycogen all decreased (P< 0.05), while muscle lactate, Cr, IMP and [H^+] increased (P<0.05). The maximal *in-vitro* K^+-stimulated 3-*O*-MFPase activity in quadriceps skeletal muscle was depressed by 13.8±4.1 % (P<0.05) at fatigue. Thus intense fatiguing exercise depresses skeletal muscle *in vitro* 3-*O*-MFPase activity. Structural alterations in the Na^+, K^+-ATPase enzyme are the most likely reason for the fatigue-induced depression in *in-vitro* 3-*O*-MFPase activity, whilst metabolic perturbations may further exacerbate the depressive effects *in-vivo*.

Study 3. No studies have examined changes in both skeletal muscle Na^+, K^+-ATPase content and activity with training. Further, no studies have examined the impact of training status on the effects of an acute exercise bout on Na^+, K^+-ATPase activity, nor the relationship between Na^+, K^+-ATPase activity with plasma [K^+]. Therefore the third study involved a cross-sectional comparison of 8 endurance trained (ET), 8 resistance trained (RT), and 8 UT subjects (Study 2), in relation to skeletal muscle 3-*O*-MFPase activity and Na^+, K^+-ATPase content. The same exercise tests, blood and muscle analyses were performed as described in Study 2. ET had a higher $\dot{V}O_2$ peak than RT

and UT (67.6±1.5, 43.8±3.6, 44.4±1.8 ml.kg^{-1}.min^{-1}, respectively P<0.05) and a lower fatigue-induced decline in quadriceps peak torque (33.3±5.3, 55.6±4.8, 49.5±2.8%, respectively, P<0.05). ET had a higher proportion of type I fibres than RT or UT (ET 67.4±3.3%, RT 43.6±4.9%, and UT 50.7±2.9%, P<0.05). ET had a 18.3 and 16.6 % higher [^{3}H]-ouabain binding site content than RT and UT respectively (ET 357±29, RT 302±52, UT 311±41 pmol.g^{-1} wet wt). There was no significant effect of training on 3-O-MFPase activity in resting muscle (ET 249± 13, RT 229± 19, UT 207± 10 nmol.min^{-1}.g^{-1} wet wt). There was a significant main effect of fatigue, with 3-O-MFPase activity decreased from 228± 9 at rest to 192± 8 pmol.min^{-1}.mg protein^{-1} at fatigue (-16.1%, P<0.05), with no differences in 3-O-MFPase activity among the groups (ET 190± 12, RT 205± 18, UT 179±9 nmol.min^{-1}.g^{-1} wet wt). $\dot{V}O_2$ peak was correlated to 3-O-MFPase activity (r = 0.46, P<0.05) and Na^+, K^+-ATPase content (r = 0.64, P<0.05) for the three groups combined. 3-O-MFPase activity was also correlated to the type I fibre percentage (r = 0.53, P<0.05). The percentage fall in 3-O-MFPase activity during MFT was correlated to the percentage decline in muscle glycogen (r=0.53, P<0.05). The lower fatigue index in ET during the MFT was related to their higher Na^+, K^+-ATPase content (r=-0.42, P<0.05). There were no differences between UT, RT, or ET in plasma [K^+] response during the MFT, but ET showed a lower $\Delta[K^+]$.work^{-1} during the $\dot{V}O_2$ peak test. 3-O-MFPase activity (r=-0.53, P<0.05) and Na^+, K^+-ATPase content (r=-0.49, P<0.05) were inversely related to $\Delta[K^+]$.work^{-1} during the $\dot{V}O_2$ peak test. In summary, the pooled 3-O-MFPase data revealed a significant depressive effect of fatigue and this was not alleviated by chronic training. The relationship between Na^+, K^+-ATPase activity and $\Delta[K^+]$.work^{-1} were inconsistent between the MFT and $\dot{V}O_2$ peak test, but they do

indicate an important role for Na^+, K^+-ATPase in regulating the exercise induced hyperkalemia.

Study 4 Lung transplantation removes the ventilatory limitation to exercise, but transplant patients (LTx) still have severely limited exercise capacity, and exhibit an excessive rise in plasma $[K^+]$.work^{-1} during exercise. The fourth study therefore explored whether impaired K^+ regulation in LTx was due to decreased muscle Na^+,K^+-ATPase content or activity. 8 LTx and 8 matched moderately active controls (CON) performed the $\dot{V}O_2$ peak test with blood analyses as described for Study 2. A resting quadriceps muscle biopsy was taken and analysed for 3-*O*-MFPase activity, Na^+, K^+-ATPase content, fibre type and metabolites. The maximal *in-vitro* K^+ stimulated 3-*O*-MFPase activity in skeletal muscle was 31 % higher (P<0.05) in LTx compared to CON, when expressed per gram wet weight (LTx: 220±15, CON: 168±9 nmol min^{-1} g^{-1} wet wt). However, despite being higher in 6 out of 8 patients, no significant difference was found when expressed relative to muscle protein content (LTx: 1426±110, CON: 1219±86 pmol.min^{-1}.mg protein^{-1}). Muscle protein was not different between LTx and CON (15.5±0.8 vs 14.9±1.0 mg.100mg^{-1}muscle wet weight). Further, there was no significant difference in Na^+, K^+-ATPase content (LTx 279±22 vs CON: 250±18 nmol.min^{-1}.g^{-1} wet weight). LTx had a higher proportion of type II muscle fibres (LTx 75.2 ±4.4% vs CON 44.6±2.1%, P<0.05), increased resting muscle lactate, $[H^+]$ and IMP, and lower ATP and AMP (P<0.05). LTx had a 51% lower $\dot{V}O_2$ peak than CON (1.21±0.08 vs 2.46±0.34 l.min^{-1}P<0.05) and displayed an earlier rise in blood [lactate] (P<0.05). The $\Delta[K^+]$ work^{-1} ratio at peak exercise in LTx was more than double the value for CON (84.5 ± 15.9 *vs* 37.1 ± 5.9 nmol.l^{-1}.J^{-1} P<0.05). Thus Na^+, K^+-ATPase content and 3-*O*-MFPase activity

are maintained or even increased in LTx and therefore these variables cannot explain the abnormal plasma [K^+] regulation during incremental exercise.

Conclusions. Utilising the modified maximal *in vitro* 3-*O*-MFPase assay optimised for human muscle, it was shown for the first time that fatigue depressed Na^+, K^+-ATPase activity in skeletal muscle. Endurance trained but not resistance trained subjects showed an increase in Na^+, K^+-ATPase content, but no groups differed in maximal *in vitro* Na^+, K^+-ATPase activity. The depressive effects of fatigue on 3-*O*-MFPase activity were not diminished in the ET or RT groups, suggesting that a depression in Na^+, K^+-ATPase activity is an obligatory response to fatigue. Na^+, K^+-ATPase activity and content were maintained in LTx but this did not prevent an abnormal [K^+] response with exercise.

Declaration

This dissertation summarises original, previously unpublished work conducted in the School of Human Movement, Recreation and Performance, and the School of Life Science and Technology at Victoria University. This dissertation is the result of work performed by the author. However considerable collaboration was also involved in the studies involving exercising humans. The muscle biopsies were conducted by qualified medical personnel. Dr Michael McKenna, Associate Prof Michael Carey and Dr Steve Selig helped in conducting the exercise tests and analyses in the biochemistry laboratory. Dr Jia Li Li and Dr Xiao Nan Wang assisted in the muscle metabolite and fibre type analyses conducted in the biochemistry laboratory.

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Publications

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Abbreviations

Subscripts		Units
i	Intracellular	
e	Extracellular	
E_m	muscle membrane potential	
Electrolytes		
K^+	Potassium ion	$mmol.l^{-1}$
Na^+	Sodium ion	$mmol.l^{-1}$
Mg^{2+}	Magnesium ion	$mmol.l^{-1}$
H^+	Hydrogen ion	$nmol.l^{-1}$
Lac^-	Lactate anion	$mmol.l^{-1}$
Ca^{2+}	Calcium ion	$mmol.l^{-1}$
HCO_3^-	Bicarbonate anion	$mmol.l^{-1}$
[ion]	ion concentration	
$\Delta[K^+].work^{-1}$	rise in $[K^+]$ relative to work done	$nmol.l^{-1}.J^{-1}$

Cardiovascular/ Blood gases

PCO_2	Partial pressure of carbon dioxide	mmHg
PO_2	Partial pressure of oxygen	mmHg
SBP	Systolic Blood Pressure	mmHg
DBP	Diastolic Blood Pressure	mmHg
HR	Heart Rate	$beats.min^{-1}$
$\dot{V}O_2$	oxygen consumption	$l.min^{-1}$

$\dot{V}O_2$peak	Peak oxygen consumption	$l.min^{-1}$
$\dot{V}CO_2$	carbon dioxide output	$l.min^{-1}$
$\dot{V}_E$	Pulmonary ventilation	$l.min^{-1}$
RER	Respiratory exchange ratio	

Muscle

Na^+, K^+-ATPase	Sodium-Potassium Adenosine Triphosphatase (EC 3.6.1.37)
Na^+,K^+-pump	Sodium-Potassium Adenosine Triphosphatase (EC 3.6.1.37)
3-*O*-MFP	3-*O*-methylfluoroscein phosphate
3-*O*-MF	3-*O*-methylfluoroscein
pNpp	*p*-nitrophenylphosphate
ATP	Adenosine 5' triphosphate
ADP	Adenosine diphosphate
IMP	Inosine monophosphate
PCr	Phosphocreatine
Cr	Creatine

Work and Power

WR	Work rate

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Chapter 1 Introduction

Tightly regulated, multitudinous cascades of events ensure the generation of muscular force necessary for humans to exercise. In skeletal muscle, depolarisation of the muscle t-tubular membrane induces Ca^{2+} release from the internal stores, the sarcoplasmic reticulum. This increases cytosolic [Ca^{2+}] and enables actomyosin crossbridge interaction and force production. The depolarisation of the sarcolemma is a consequence of the movement of sodium (Na^+) into the cell, whilst repolarisation occurs with potassium (K^+) ion efflux from the cell. To maintain excitability, the steep Na^+ and K^+ concentration gradients are restored via the activation of the Na^+,K^+-ATPase enzyme (EC 3.6.1.37), otherwise known as the Na^+,K^+-pump (Clausen 1986; Nielsen & Overgaard 1996). Maintenance of Na^+ and K^+ gradients via Na^+, K^+-ATPase activity/activation is critical for muscular function to prevent the sarcolemmal membrane from becoming further depolarised, possibly leading to T-tubular block of action potential (AP) propagation. This AP block would abolish cell activation and induce complete loss of contractile force in that fibre leading to reduced muscular performance (Hanson & Person 1971).

As skeletal muscle has the largest pool of K^+ in the body (Clausen & Everts, 1989), skeletal muscle reaccumulation of [K^+] is critical to whole body [K^+] regulation. During exercise, not all of the K^+ exiting the contracting fibres is reaccumulated via the Na^+, K^+-ATPase, and consequently muscle interstitial and plasma [K^+] rises. The relationship between Na^+, K^+-ATPase content or activity and plasma [K^+] responses in exercising humans has been investigated in very few studies (Green et al. 1993; Kjeldsen 1990b; McKenna et al. 1993). These studies showed that training-induced increases in Na^+, K^+-ATPase content did not correlate with reduced plasma [K^+] during exercise. No studies have comprehensively examined Na^+, K^+-ATPase activity and content in human skeletal

muscle in relation to acute exercise, training, chronic inactivity or $[K^+]$ response during exercise.

For complete functional determination of the Na^+, K^+-ATPase, both content and activity should be measured. Previously, only content has been measured in human skeletal muscle and therefore the possibility of differential changes in content or activity cannot be determined. This is important, as studies may find no change in content of Na^+, K^+-ATPase but a possible activity change may go undetected (Druml et al. 1988; Pickar et al. 1994). This thesis measured Na^+, K^+-ATPase activity using a modified and optimised K^+-stimulated 3-*O*-MFPase activity and measured Na^+, K^+-ATPase content via $[^3H]$-ouabain binding. This maximal K^+-stimulated phosphatase activity represents the terminal phosphatase reaction in the process of ATP cleavage, Na^+ extrusion, and K^+ reaccumulation in the Na^+, K^+-ATPase cycle (Elmosehli et al. 1994). Thus the assay does not indicate the physiological activity *in vivo*, but rather measures the *in vitro* maximal K^+-stimulated phosphatase activity. The effects of human muscle fatigue on Na^+, K^+-ATPase activity, and the relationship to plasma of $[K^+]$ was examined. Further, an activity continuum, ranging from chronic muscle disuse in lung transplant recipients to long-term endurance or resistance trained individuals was used to investigate differences in Na^+, K^+-ATPase function and plasma $[K^+]$ responses.

Chapter 2 Literature Review

2.0 Historical overview of the Na^+, K^+-ATPase and ionic regulation

The question of how cells maintain a relatively low [Na^+] and high [K^+], while surrounded by interstitial fluid with a reversed concentration ratio of these cations has been investigated for over 100 years. Fenn & Cobb (1936) noted that during electrical stimulation, rat and frog muscle gained Na^+ and lost K^+ and these movements reversed following electrical stimulation. Another early study in dogs found a rapid uptake of labelled $^{22}Na^+$ into red blood cells, indicating membrane permeability to Na^+ (Cohn & Cohn 1939). One of the earliest studies in humans reported that venous [K^+] increased 25% during short duration exhaustive exercise and that it fell precipitously in recovery (Keys 1937). Thus it was clear that Na^+ and K^+ flowed across the muscle membrane during muscle excitation and these movements reversed following electrical stimulation. The concept of active transport developed in the 1940's and 1950's (Skou 1998). The 'pump-leak concept' was developed where Na^+ and K^+ leaked down their electrochemical gradients but K^+ had to be actively pumped into the cell against its concentration gradient (Dean 1941; Skou 1957, 1998). Despite this prevailing concept, the identity of this pump system and its relationship to cell metabolism was not clear.

Dean proposed a membrane pump theory in 1941, and from this work he concluded that "the muscle can actively move potassium and sodium against concentration gradients

...(but) this requires work. Therefore there must be some sort of a pump, possibly located in the fibre membrane, which can pump out sodium or, which is equivalent, pump in the potassium." It was subsequently shown that ATP was required for this transport and that the membrane transport pump could be poisoned by a cardiac glycoside (Hodgkin & Keynes 1956; Schatzmann 1953). The work of Jens Christian Skou identified this membrane transport pump. Skou fractionated a crab nerve homogenate into a microsomal component, which exhibited ATPase activity when stimulated by Na^+, K^+, and Mg^{2+} (Skou 1957). He conversed with Robert Post who indicated that the stoichiometry of Na^+ extrusion out of and K^+ entry into red blood cells was in the ratio of 3:2 (Post & Jolly, 1957; Post et al. 1957). Skou later reasoned that the previous identification of a cardiac glycoside (ouabain) suppressible cation pump (Schatzmann 1953) might indeed be the Na^+, K^+-ATPase enzyme (Skou 1960). His hypothesis was further supported in subsequent publications, and Skou summarised the active transport of Na^+ and K^+ via the enzyme system in his classic 1965 review. In the following decade, his work was confirmed in a number of laboratories, with the importance of Skou's work recently recognised with the 1997 Nobel Prize for Chemistry (see Clausen & Persson 1998).

Between 1957 and the present day, research into the Na^+, K^+-ATPase enzyme kinetics, structure, and molecular biology has generated over 10,000 papers (Clausen & Persson 1998). However there is a comparative lack of research on Na^+, K^+-ATPase function in humans (Clausen 1998). Overall, animal and human research has improved our understanding of the physiological role of the Na^+, K^+-ATPase enzyme in various tissues, including its role in skeletal muscle excitation, fatigue and training.

The Na^+, K^+-ATPase enzyme was first demonstrated in human skeletal muscle membrane fractions obtained from cadavers (Samaha & Gergely 1965, 1966). Samaha & Gergely and many of the earlier researchers attempted to identify the Na^+, K^+-ATPase in various tissues (Matsui & Schwartz 1966; Samaha & Gergely 1965, 1966; Sulakhe et al. 1971) using purification techniques to eliminate many of the unspecific ATPases found in skeletal muscle. The use of the vanadate-facilitated [^{3}H]-ouabain binding technique enabled accurate quantification of Na^+, K^+-ATPase content in muscle biopsies or intact muscle fibres (Nørgaard et al. 1983, 1984a). This technique also avoided the problems associated with purification of sarcolemmal components (Hansen & Clausen 1996). Sensitive fluorescent techniques have been used to measure Na^+, K^+-ATPase activity in rodent muscle (Nørgaard et al. 1984b). The measurement of ouabain-suppressible K^+ influx or Na^+ efflux across the sarcolemma of isolated intact muscle preparations have also been used to quantify Na^+, K^+-ATPase transport rate (Clausen et al. 1987; Hansen & Clausen 1988). Analogues of K^+ have also been used including ^{42}K and rubidium (^{86}Rb, Clausen et al. 1987).

The extent of Na^+, K^+-ATPase activation during muscle contractions can be estimated via arterio-venous [K^+] differences across contracting muscle (Hallén et al. 1994; Juel et al. 1990; 1999; McKenna et al. 1997; Sjøgaard et al. 1985; Verburg et al. 1999; Vøllestad et al. 1994). There have also been attempts to link the functional consequences of alterations in Na^+, K^+-ATPase activity or content. The relationship between Na^+, K^+-ATPase and the plasma [K^+] response during exercise has been explored (Green et al. 1993; Kjeldsen et al. 1990b; Klitgaard & Clausen 1989; Madsen et al. 1994; McKenna et al. 1993). Some of these studies have shown an increase in Na^+, K^+-ATPase content after

training and a reduced hyperkalemia during exercise, but these variables were not significantly correlated (Green et al. 1993; Madsen et al. 1994).

The loss of K^+ from contracting skeletal muscle was proposed to be due to inadequate Na^+, K^+-ATPase capacity and to be of importance in limiting contractile performance (Clausen 1990). This idea was given credence by researchers such as Sjøgaard (1990), Balog & Fitts (1996), Jones (1996), and Nielsen & Clausen (2000).

Research over the past three decades has convincingly shown that the Na^+, K^+-ATPase enzyme is subject to both acute and long-term regulation (Clausen 1986). Important information gained includes the stimulatory effect of numerous hormones and other agents, possible translocation from intracellular stores to the sarcolemma, and the effects of physical training or detraining (eg Clausen 1996a; Green et al. 1993; Juel et al. 2000a; Lavoie et al. 1996; McKenna 1998). Molecular biology techniques have clarified some the various Na^+, K^+-ATPase isoform expression and their tissue-specific functions in both animal and human tissues (Hundal et al. 1993; Juel et al. 2000a; Lavoie et al. 1996; Sweadner 1979).

In 1940 Fenn noted 'We are still far from being in a position to present the known facts concerning the physiology of potassium from the point of view of any well developed theory of potassium behaviour". This statement still has relevance today, despite 70 years of active research in potassium and Na^+, K^+-ATPase regulation, many questions still remain unclear or unanswered. Notably the role of extracellular K^+ and Na^+, K^+-ATPase function in muscle fatigue, and exercise training are not yet fully understood. This dissertation will examine Na^+, K^+-ATPase and its role in muscle fatigue, the effects of training and chronic detraining, and its relationship with plasma $[K^+]$ during and following exercise.

2.1 Structure/ Function of the Na^+, K^+-ATPase

2.1.1 Na^+, K^+-ATPase function

The Na^+, K^+-ATPase transports Na^+ and K^+ ions against their transmembrane concentration gradients and, in the case of Na^+, against an electrical gradient as well. For human skeletal muscle the extracellular [Na^+] and [K^+] have been measured at ~134 and ~4.6 mmol l^{-1} respectively and the intracellular ion concentrations at ~9 mmol l^{-1} for [Na^+] and ~160 mmol l^{-1} for [K^+] (Sjøgaard 1983). Recently, interstitial [K^+] has been measured in resting human muscle as 3.9-4.3 mmol l^{-1} using the microdialysis technique (Green et al. 1999b, Juel et al. 2000b). The above result for interstitial [K^+] is lower than the Sjøgaard (1983) reported value, but it must be kept in mind that Green et al. 1999b and Juel et al. 2000b analysed interstitial [K^+] using different techniques. Importantly the interstitial values reported during knee extensor exercise are much higher than published femoral venous [K^+] (Bangsbo et al. 1996; Juel et al. 2000b). The [K^+] response to exercise will be discussed further in 2.4.2.

The potential energy of the ionic chemical gradients maintained by the Na^+, K^+-ATPase provides the driving force for the transport of other solutes, notably amino acids, creatine, glucose and phosphate (Ewart & Klip 1995; Sweeney & Klip 1998). This potential energy from the ionic gradients (namely Na^+) also allows the removal of protons, calcium, and bicarbonate (Lechene 1988). It appears that the Na^+, K^+-ATPase is exquisitely regulated with changes in Na^+ influx, that is, the leak of Na^+ and the maintenance of this ionic gradient by the Na^+, K^+-ATPase drive many co- and counter transport mechanisms. ATP hydrolysis is linked to the exchange of these ions where 3 Na^+ ions are extruded and 2 K^+ ions are reaccummulated back into the cell per ATP

molecule split. A considerable amount of energy derived from cytosolic ATP breakdown is required to maintain these ion gradients and cellular processes. By restoring Na^+ and K^+ against their skeletal muscle membrane gradients, the Na^+, K^+-ATPase modulates muscle membrane excitation and contraction.

2.1.2 Na^+, K^+-ATPase structure

The nomenclature for the sodium-potassium-adenosine triphosphatase (Na^+, K^+-ATPase) enzyme (EC 3.6.1.37) varies in the literature: Na^+, K^+-pump, Na^+, K^+-ATPase, sodium pump, but will be referred to in this thesis as Na^+, K^+-ATPase. The Na^+, K^+-ATPase enzyme is a transmembranous protein situated in the lipid bilayer of virtually all excitable animal cells. This transmembranous protein actively extrudes three Na^+ ions from cells and imports two K^+ ions into the cell, coupled to the hydrolysis of one ATP molecule. It is also referred to as the Na^+, K^+-pump as it pumps Na^+ and K^+ ions against their concentration gradients. The enzymatic structure (Figure 2.1) comprises a catalytic α subunit (molecular weight ~112kDa) which may span the membrane 10 times and a single span glycosylated β subunit (~55kDa, Fambrough et al. 1994; Lavoie et al. 1997). A third protein, termed the γ-subunit is a small hydrophobic polypeptide of 8-14kDa but its functional role is unclear (Blanco & Mercer 1998). The α subunit contains the ligand (Na^+, K^+, Mg^{2+}, ATP, and P*i*) binding sites as well as sites for the specific inhibitors, ouabain and digoxin, which bind to the extracellular surface (Jorgensen 1982). Several studies have shown that by combining with the α subunit, the β subunit is required for normal function and it may act as a chaperone, stabilising the correct folding of the subunit to facilitate its delivery to the plasma membrane (Blanco & Mercer 1998; McDonough et al. 1990; Sweadner, 1989). There is also the suggestion that the β subunit acts as an adhesion molecule, interacting with receptors on adjacent cells, guiding the

Na^+, K^+-ATPase to specific regions of the plasma membrane (Ackermann & Geering 1990; Ewart & Klip 1995). These subunits appear as a functional αβ complex, which is the minimum functional unit to form the Na^+, K^+-ATPase ion transporter (Jorgensen 1982, Levenson 1994; Lingrel 1992).

2.1.3 Location and content of Na^+, K^+-ATPase

The Na^+, K^+-ATPase is oriented in the membrane with part of the protein exposed at both faces (Fig 2.1). The region of the protein that faces the cytoplasm has three high-affinity sites for binding Na^+ ions and one site for an ATP molecule. On the outer face the enzyme has two high-affinity binding sites for K^+ ions as well as a binding site for ouabain and digoxin: cardiac glycosides that inhibit Na^+, K^+-ATPase (Jorgensen 1982; Rouho & Kyte 1974). Both ouabain and digoxin are cardiac glycosides which affect cardiac muscle by increasing vagal activity and slowing the rate of conduction through the AV node. The force of cardiac contraction is also increased as the binding of these glycosides to the Na^+, K^+-ATPase causes intracellular [Na^+] to rise and the cardiac muscle cell becomes depolarised. The raised intracellular [Na^+] exchanges with Ca^{2+} through Na^+ /Ca^{2+} exchanger resulting in increased intracellular [Ca^{2+}] and force production. Both ouabain and digoxin can inhibit Na^+, K^+-ATPase (by binding to the K^+ binding site) in cardiac as well as skeletal muscle.

In skeletal muscle the Na^+, K^+-ATPase is located in the sarcolemma (Sulakhe et al. 1971) as well as in the transverse tubules (Lau et al. 1977). There is also evidence for an intracellular pool that can translocate to the plasma membrane upon hormonal and exercise stimulation (Juel et al. 2000a; Lavoie et al. 1996; Omatsu-Kanbe & Kitasato 1990; Tsakirides et al. 1996). The sarcolemmal Na^+, K^+-ATPase content, as estimated from [^{3}H]-ouabain binding, ranges from 1000 μmol.m^{-2} in guinea pig muscle (Harrison et al. 1994) to 3500 μmol.m^{-2} in rat soleus muscle (Clausen & Hansen 1974; Erlij &

Grinstein 1976). The t-tubules represent up to 70% of the surface area (Eisenberg & Kuda 1976), but Na^+, K^+-ATPase content measurements performed on frog muscle detubulated by glycerol pretreatment, showed that only ~20% of the total number of Na^+, K^+-ATPase are located in the transverse tubules (Venosa & Horowitz 1981). Studies on Na^+, K^+-ATPase in t-tubule of mammalian muscle do not state the relative difference in Na^+, K^+-ATPase expressed in t-tubules versus sarcolemma (Ariyasu et al. 1987; Horgan & Kuypers 1988). In spite of the much larger surface area, the transverse tubules contain only a minor proportion of Na^+, K^+-ATPase in skeletal muscle. The low density of Na^+, K^+-ATPase in the t-tubule combined with a smaller distribution volume for the released K^+ than outside the sarcolemma, may predispose this site to action potential block, and failure of transmission during muscle contraction. This may have important consequences for fatigue especially during high intensity exercise (Balog & Fitts 1996; McKenna 1998).

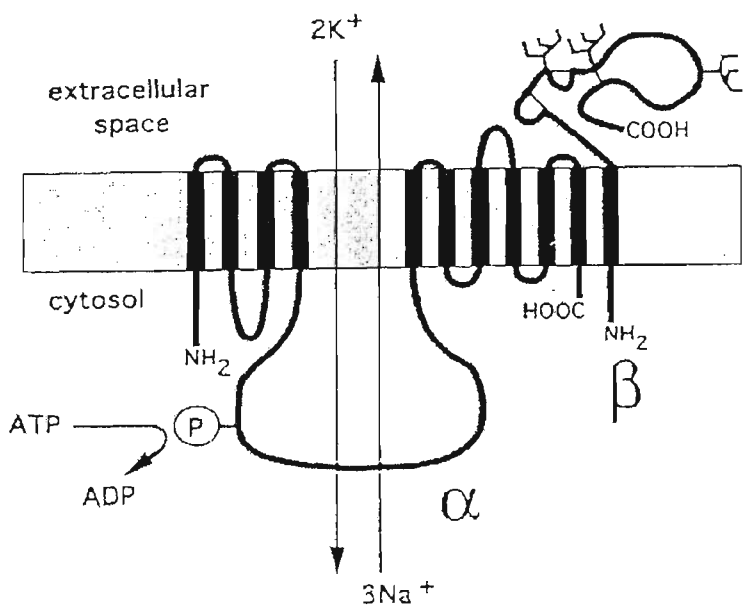

Figure 2.1 Model of the Na^+, K^+-ATPase structure. The α subunit spans the membrane 10 times and while the glycosylated β subunit has a single span. (P denoted phosphorylation). From Fambrough et al. (1994).

2.1.4 Isoforms of the Na^+, K^+-ATPase

Since Sweadner (1989) identified 2 isoforms of Na^+, K^+-ATPase in rat brain, there has been considerable effort in identifying the various isoforms of the Na^+, K^+-ATPase. The isoform expression is tissue-, organ- and maturation-dependent (Herrera et al. 1994; Levenson 1994; Munzer et al. 1994) and can be detected by specific antibodies (Lavoie et al. 1996; Sweadner 1993). The α and β subunits exist in three isoforms (α_1, α_2, α_3, β_1, β_2, and β_3) with all except β_3 expressed in mammalian skeletal muscle. An α_4 isoform has also been identified in mammalian cells (Blanco & Mercer 1998; Shamraj & Lingrel 1994). Human skeletal muscle express α_1, α_2, and α_3, but their catalytic activity is only supported by the β_1 isoform (Hundal et al. 1994; Juel et al. 2000a). Tissues vary in their complement of α and β subunits, with α_1 present in most plasma membranes, α_2 present in muscle, heart, adipose tissue and brain, and α_3 predominantly present in heart and brain. The β_1 isoform is present in most cells except fast twitch glycolytic muscle fibres, which only express the β_2 isoform (Hundal et al. 1993). Our understanding of the physiological significance of why certain tissues such as brain, muscle and heart express different isoform subunits in the same cell is still evolving, but it may be related to the type of regulation that each can experience (Ewart & Klip 1995).

2.1.5 Isoform locations in skeletal muscle

Recent studies using molecular biology techniques, involving skeletal muscle membrane purification and labelling with specific antibodies, have shown various locations for the isoforms of the Na^+, K^+-ATPase. Before discussing these isoforms of the Na^+, K^+-ATPase it is important to note that often less than 1% of the plasma membrane is recovered in these studies, so some of the interpretations may be misleading (Hansen & Clausen 1996). Hansen & Clausen (1996) noted that many studies involving isolation

procedures use a calculation with a purification factor and do not compare results to that of intact tissue. The assumption from many studies that the final product is sarcolemmal in origin and representative of a significant fraction of this structure can also be questioned. Accepting these limitations with regard to the use of enzyme activities for calculation of sarcolemmal recoveries, Hundal & Aledo (1996) suggest that this low yield fraction does provide useful information about membrane proteins. They support the use of these purification steps by showing that immunological assays with antibodies to specific membrane proteins show that they are present in the fraction termed plasma membrane. The results of some of these purification studies are presented below. In rat skeletal muscle the α_1 subunit was almost exclusively found in the sarcolemma (Hundal et al. 1992, 1993) while α_2 subunits were found in the sarcolemma but also in t-tubules and intracellular membrane fractions (Marette et al. 1993). As with the α_2 isoform, the β_1 subunits have been reported at both intracellular sites and at the surface membrane (Hundal et al. 1992, 1993). Due to the large amount of tissue required for purification of membrane fractions, studies on human tissue have been limited. Hundal and colleagues (1994) overcame these difficulties by accessing 20-30g of human soleus muscle from amputated limbs. They noted that the α_1 was found mainly in the sarcolemma in human skeletal muscle, while the α_2 isoform was located more in intracellular membrane fractions. They also noted that human skeletal muscle was distinct from rat tissue in that the α_3 isoform was also expressed. The β_2 isoform is not present in human skeletal muscle and the β_1 isoform is mainly located at the plasma membrane (Hundal et al. 1994). Juel et al. (2000a) confirmed the findings of Hundal et al. (1994) using the sarcolemmal giant vesicle technique. Juel et al. (2000a) showed that only the β_1 isoform was present in sarcolemma of human skeletal muscle and the α_1 isoform was primarily located at the plasma membrane. They also found that the α_2 subunit content was

increased in the sarcolemma after exercise and the α_1 subunit unchanged between plasma membrane and muscle homogenate measures. Juel et al. (2000a) concluded that the α_2 subunit may serve as a pool of subunits available for translocation. Therefore in human skeletal muscle, the α_1, α_2, and α_3, combine with the β_1 isoform to form the functional heterodimer.

2.1.6 Isoform function

In human muscle the common isoforms display similar affinities for ATP, cytoplasmic Na^+ and extracellular K^+, but some isoform-specific differences in affinity have been observed (Blanco & Mercer 1998; Lingrel et al. 1998; Munzer et al. 1994). The following table summarises some of these isoform specific affinities for various substrates.

Table 2.1 Kinetic characteristics of rat Na^+, K^+-ATPase isoform combinations (modified from Blanco & Mercer 1998, $K_{0.5}$: concentration required for half maximal activity, K_i: concentration required for complete ouabain inhibition).

isoform	Na^+ Activation $K_{0.5}$, mM	K^+ Activation $K_{0.5}$, mM	ATP Activation $K_{0.5}$, mM	Ouabain Inhibition K_i, mM
$\alpha_1\beta_1$	16.4 ± 0.4	1.9 ± 0.2	0.46 ± 0.10	4300 ± 1900 x 10^{-8}
$\alpha_2\beta_1$	12.4 ± 0.5	3.6 ± 0.3	0.11 ± 0.01	17 ± 1.0 x 10^{-8}
$\alpha_3\beta_1$	27.9 ± 1.3	5.3 ± 0.3	0.09 ± 0.01	3.1 ± 0.3 x 10^{-8}
$\alpha_2\beta_2$	8.8 ± 1.0	4.8 ± 0.3	0.11 ± 0.02	15 ± 2.0 x 10^{-8}
$\alpha_3\beta_2$	17.1 ± 1.0	6.2 ± 0.4	0.07 ± 0.02	4.7 ± 0.4 x 10^{-8}

These isoforms have similar affinities for ouabain except for rat α_1 which is much lower than α_2 and α_3 (Lingrel et al. 1998). The α_1 isoform has been suggested to be a "housekeeping" form of the Na^+, K^+-ATPase which is capable of responding to typical physiological demands (Munzer et al. 1994). Changes in Na^+, K^+-ATPase isoform expression in different tissues, subject to differences in age, electrolytes, and hormonal condition could have important physiological effects (Hansen 1998). Indeed the molar ratios of β to α subunits may serve to regulate the catalytic activity of the Na^+, K^+-ATPase (Lavoie et al. 1997).

2.1.7 Translocation of Na^+, K^+-ATPase subunits to the sarcolemma

Several studies have provided evidence that the location of Na^+, K^+-ATPase isoforms is not fixed, but rather subunits can be recruited from an intracellular pool to the plasma membrane, under the stimulation of insulin (Lavoie et al. 1996; Omatsu-Kanbe &

Kitasato 1990) or even exercise (Juel et al. 2000a; Tsakirides et al. 1996). It is likely that an increased number of functional Na^+, K^+-ATPase units at the plasma membrane increase Na^+, K^+-ATPase activity at this site, analogous to the insulin-induced translocation and increased activity of GLUT4 transporters in the plasma membrane (Hundal et al. 1992; Lavoie et al. 1996; Sweeney & Klip 1998). There is also evidence that the translocation occurs mainly in slow twitch fibres and that this is mediated by insulin (Lavoie et al. 1996; Marette et al. 1993). In rat soleus muscle, *in vivo* insulin stimulation resulted in 80% and 124% increases in the expression of α_2 and β_1 isoforms at the plasma membrane in oxidative fibres, as assessed by subcellular muscle fractionation and immunoblotting with isoform specific antibodies (Lavoie et al. 1996). However it must be acknowledged that these studies have not used a quantitative approach and the magnitude of this proposed effect has not yet been determined. Also, early studies reporting translocation of Na^+, K^+-ATPase subunits with insulin were later criticised as simply reflecting an increased ouabain binding rate in insulin-stimulated muscles (Clausen & Flatman 1987). Therefore further studies are required to confirm this insulin mediated translocation of Na^+, K^+-ATPase subunits. Finally it could be argued that insulin and exercise stimulate Na^+, K^+-ATPase subunit translocation via different mechanisms as plasma insulin levels fall during exercise. However there is increased muscle blood flow (Delp 1998) and skeletal muscle has an increased sensitivity to insulin, thus total insulin delivery to muscle is increased with exercise (Araujo-Vilar et al. 1997).

2.2 Mechanism of action of Na^+, K^+-ATPase

The specific regions on the catalytic α subunit have been studied recently using techniques such as chemical modification (Arguello & Kaplan 1994), proteolytic digestion, and site-directed mutagenesis (Jorgensen et al. 1998). These studies indicate that the cytoplasmic loop between transmembrane segments M4 and M5, and the specific

amino acids therein, are important in the regulation of ATP hydrolysis and its modifications by cations (Kaplan et al. 1998). Prior to binding the cations in the cation binding, domain are sensed in the ATP binding domain (Kaplan et al. 1998), indicating that the coupling of cation binding and ATP hydrolysis is well regulated. However, the details of the mechanisms of coupling between the Na^+ and K^+ transport reactions and ATP cleavage remains relatively unknown. This implies that a great deal of research has investigated the functional role the Na^+, K^+-ATPase, but much less research has focussed on how the Na^+, K^+-ATPase actually works (Apell et al. 1998). The following is a modified schema from Apell et al. (1998) divided into several steps (Fig 2.2). Firstly with the Na^+, K^+-ATPase protein in one conformational state, termed E_1, an ATP molecule and three Na^+ ions bind to their respective sites on the cytoplasmic inner surface of the cell membrane (step 1-3 on Fig 2.2). In a reaction that requires a magnesium (Mg^{2+}) ion tightly complexed to the ATP (step 4 on Fig 2.2), the bound ATP is hydrolysed to ADP, while the liberated phosphate is transferred to an aspartate residue in the Na^+, K^+-ATPase, forming a high energy acyl phosphate bond. Once the ADP is released (step 5 on Fig 2.2) the protein then changes its conformation to another state termed E_2. Then three Na^+ ions are propelled out of the cell (steps 6-8 on Fig 2.2). Two K^+ ions subsequently bind to the extracellular face of the protein (step 9-10 on Fig 2.2) and the acyl phosphate is hydrolysed to aspartate and free phosphate (step 11-12 on Fig 2.2). Steps 9-12 are proposed to be the K^+-dependent phosphatase activity of the Na^+, K^+-ATPase enzyme and assays such as the 3-*O*-MFPase are reported to represent this activity (Askari & Koyal 1968; Horgan & Kuypers 1987; Robinson and Flashner 1979). Ouabain also binds to the extracellular face of the enzyme and competes with K^+ for the same binding site (Hansen 1982). Finally the enzyme returns to its original conformation (step 13 on Fig 2.2), and with the binding of ATP, the two K^+ ions are released into the

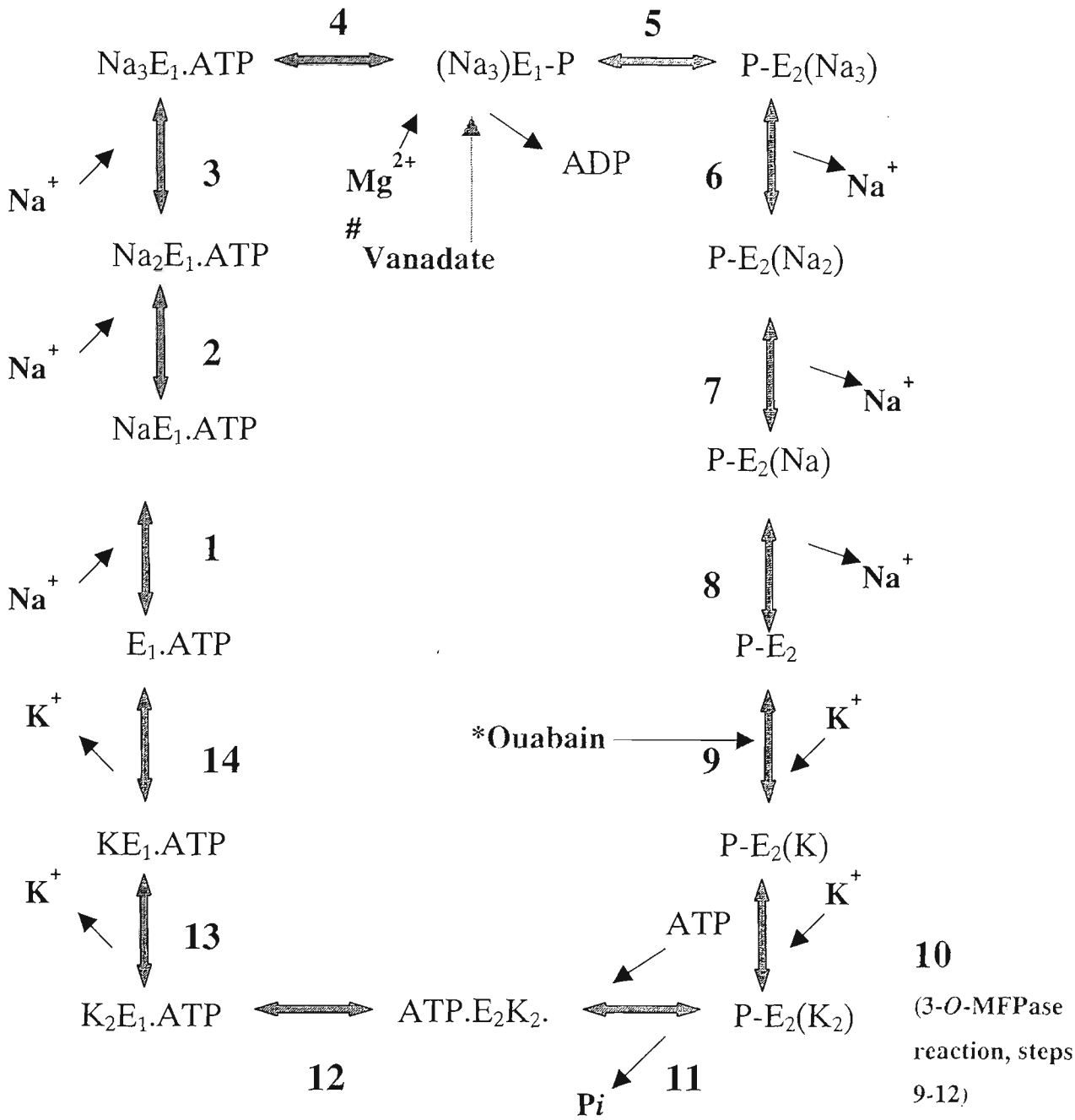

Figure 2.2. Scheme for mechanism of action of the Na^+, K^+-ATPase. Modified from Apell et al. (1998) & Johnson & Chapman (1985). #vanadate binds to the phosphorylation site on the inner surface, *ouabain binding tightly to inner surface of enzyme at the K^+ binding site . For [^{3}H]-ouabain, vanadate binds to the inner surface of the membrane at the phosphorylation site. This is also the K^+ binding site which is usually intracellular but accessible when homogenate vesicle are broken up by detergent treatment of freeze fracture. See text for details.

cell (step 13-14 on Fig 2.2). The net result is the hydrolysis of one ATP, and release of one ADP and P*i*, extrusion of 3 Na^+ ions and uptake of two K^+ ions.

2.3 Quantification of Na^+, K^+-ATPase content and activity

Various methods are available for studying skeletal muscle Na^+, K^+-ATPase, including measuring of content using [^{3}H]-ouabain binding site content, or estimates of Na^+, K^+-ATPase activity utilising related phosphatase activity, or inorganic phosphate (P_i) production (Akera 1984; Nørgaard et al. 1984a, 1984b). The above measures are often used to quantitate Na^+, K^+-ATPase content, with activity measures being converted to content by assuming a certain molar activity for the Na^+, K^+-ATPase enzyme (Nørgaard et al. 1984b). Other measures used to estimate Na^+, K^+-ATPase activity *in-vivo* include the measurement of arterio-venous [K^+] differences across an exercising muscle bed or limb (Hallén et al. 1994; Juel et al. 1990; McKenna et al. 1997; Verburg et al. 1999; Vøllestad et al. 1994). The following sections outline the most commonly used methods to determine Na^+, K^+-ATPase content and activity in skeletal muscle.

2.3.1 Na^+, K^+-ATPase content

Complete recovery of the Na^+, K^+-ATPase enzyme is a prerequisite for quantitative measurements (Kjeldsen et al. 1988). The low recovery of enzyme activity with purification techniques can be avoided by measuring the binding of the radioactively labelled digitalis glycoside [^{3}H]-ouabain to Na^+, K^+-ATPase in the plasma membrane of skeletal muscle (Kjeldsen et al. 1984a, Nørgaard et al. 1984a). This technique measures all functional Na^+, K^+-ATPases as incubation in a buffer containing a saturating concentration of ouabain allows each Na^+, K^+-ATPase to tightly and specifically bind one

molecule of [^{3}H]-ouabain (Clausen 1990). By measuring the amount of bound [^{3}H]-ouabain, the total content of functional Na^+, K^+-ATPase can be readily determined.

Ouabain binding is a reversible second order reaction that requires ATP or other nucleotides, utilising Mg^{2+} as a co-factor, and is stimulated by Na^+ but inhibited by K^+ (Nørgaard 1986). Ouabain binding was initially performed on intact muscles or *in vivo* utilising ATP to promote the binding of [^{3}H]-ouabain to Na^+, K^+-ATPase (Hansen 1979). As the content of Na^+, K^+-ATPase is relatively low in skeletal muscle compared to other tissues, vanadate (VO_4, a phosphate (PO_4) analog) has been used to promote binding of the glycoside to the receptor (Hansen 1979; Nørgaard et al. 1983). Vanadate, in the presence of Mg^{2+} binds to the phosphorylation site on the inner surface of the plasma membrane, and promotes high affinity ouabain binding on the plasma membrane (Hansen 1982). As a stoichiometric relationship exists between ouabain binding capacity and hydrolytic activity of the Na^+, K^+-ATPase (Hansen 1971), and identical binding capacities exist for ATP, ADP and ouabain (Hansen et al. 1971), [^{3}H]-ouabain binding can used for the quantification of functional Na^+, K^+-ATPase. In dissected muscle pieces, such as those obtained from muscle biopsy samples, vanadate ensures binding of [^{3}H]-ouabain to all functional Na^+, K^+-ATPase (Clausen 1996a; Nørgaard et al. 1983). The [^{3}H]-ouabain binding site content can be expressed as the number of Na^+, K^+-ATPase per gram of tissue wet weight or more commonly, as picomoles per gram tissue wet weight. In untrained human skeletal muscle, Na^+, K^+-ATPase contents of approximately 300 pmol g^{-1} wet wt have been reported (Dørup et al. 1988; Klitgaard & Clausen 1989, McKenna et al. 1993; Nørgaard et al. 1984a; Nørgaard 1986).

2.3.2 Na^+, K^+-ATPase activity

The capacity to reduce the net loss of K^+ and gain in Na^+ from skeletal muscle depends on the total content, as well as the maximal activity/extent of activation of the Na^+, K^+-ATPase. Although most authors have focussed on factors regulating the total content of Na^+, K^+-ATPase in muscle, it is apparent that under certain conditions, enzyme activity may be altered independently from the total content (Druml et al. 1988; Pickar et al. 1994). For example, Druml et al. (1988) showed that while basal Na^+, K^+-ATPase activity was decreased by 30% in skeletal muscle of rats with chronic renal failure, Na^+, K^+-ATPase content was not affected. They concluded that Na^+, K^+-ATPase turnover rate was decreased. Pickar et al. (1994) showed that spontaneously hypertensive rats had increased soleus muscle Na^+, K^+-ATPase content, but lower Na^+, K^+-ATPase activity than control rats. Thus, to fully quantitate muscle maximal Na^+/K^+ exchange capacity it is necessary to measure both Na^+, K^+-ATPase activity and the total content of Na^+, K^+-ATPase.

A common method for determining Na^+, K^+-ATPase activity is through measurement of radiolabelled ion fluxes, with this technique being applied during electrical stimulation of isolated intact muscle preparations (Clausen et al. 1987). Despite their widespread use, these techniques cannot be used to measure Na^+, K^+-ATPase activity in human skeletal muscle samples obtained by needle biopsy. Biochemical methods used to quantify the maximal *in-vitro* Na^+, K^+-ATPase activity in skeletal muscle samples include measurements of inorganic phosphate production in purified preparations (Bonting 1971), or the activity of related K^+-dependent phosphatases, using substrates such as *p*-nitrophenyl phosphate (pNpp, Hundal et al. 1994; Judah et al. 1962), or 3-*O*-methylfluorescein phosphate (3-*O*-MFP, Kjeldsen et al. 1988). The use of purification procedures inevitably results in extremely low recovery of enzyme activity and therefore

has considerable limitations when used for quantification of total Na^+, K^+-ATPase activity (Hansen & Clausen 1996; Huang & Askari 1975; Nørgaard et al. 1984b). In contrast, complete membrane and enzyme recovery can be attained using the K^+-stimulated pNppase and 3-*O*-MFPase assays in rat muscle homogenates (Hansen & Clausen 1996). The K^+-stimulated 3-*O*-MFPase assay has a 2-3 times higher sensitivity, therefore requiring 50-100 times less tissue than the K^+-stimulated pNppase (Nørgaard 1986). Thus, the K^+-stimulated 3-*O*-MFPase assay is the preferred method for studies investigating Na^+, K^+-ATPase activity in muscle homogenates from small muscle biopsy samples and allows for complete recovery of enzyme activity.

2.3.3 3-*O*-MFPase activity

The activity of the K^+ dependent 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase), an enzyme performing part of the Na^+, K^+-ATPase reaction, can be determined at optimal conditions on crude muscle homogenates (Askari & Koyal 1968; Benders et al. 1992; Nørgaard et al. 1984b). This highly sensitive fluorimetric assay allows for the determination of the hydrolytic activity of the Na^+, K^+-ATPase in preparations with low activity (crude homogenates), thereby avoiding complications due to isolation of the enzyme and ensuring that the total enzyme activity in the tissue is measured (Nørgaard et al. 1984b). Nørgaard et al. (1984b) reported maximal K^+-stimulated 3-*O*-MFPase activity values of 190-370 $pmol.min^{-1}.g^{-1}$ wet wt in rat skeletal muscle with values decreasing with age and K^+ deficiency. In human skeletal muscle Benders et al. (1992) reported maximal K^+-stimulated 3-*O*-MFPase activity values of 382 $nmol\ min^{-1}\ g^{-1}$ protein or 57 $pmol.min^{-1}.g^{-1}$ wet wt (assuming 15% protein). Therefore it is clear that human skeletal muscle has a lower inherent Na^+, K^+-ATPase activity compared to rat skeletal muscle.

2.4 Muscle ionic fluxes with exercise

2.4.1 Membrane activation

The most important ions influencing the resting membrane potential (resting E_m) are chloride (Cl^-), Na^+, and K^+ (Cunningham et al. 1971; Dean 1941). These ions develop a transmembranous electrochemical charge due to their separation across the membrane (Cunningham et al. 1971; Hodgkin & Huxley 1952). In a resting state, the cell is polarised with the potential difference across the membrane at rest being approximately –90mV in human skeletal muscle (Cunningham et al. 1971; Sjøgaard et al. 1985). The resting E_m is calculated from the individual permeabilities and the sarcolemmal differences in chemical potential of the charged ions (Hodgkin & Horowitz, 1959).

Propagation of an action potential in neurons in order to excite a muscle fibre depends essentially on the difference in membrane permeability to Na^+ and K^+ and the maintenance of steep chemical gradients for Na^+ and K^+. During the action potential, E_m increases rapidly from –90 to $^+$40mV due to the opening of voltage-gated Na^+ channels resulting in a large increase in membrane permeability to Na^+ (Ruff et al. 1996).

2.4.2 Muscle Na^+ and K^+ fluxes

With each action potential, there is a net Na^+ influx and K^+ efflux (Creese et al. 1958; Clausen & Everts 1989; Everts & Clausen 1988). Reversing the flow of these ions is achieved via the activation of the Na^+, K^+-ATPase (Clausen, 1986). Despite this Na^+, K^+-ATPase activation, a net K^+ efflux of about 9.6 pmol.cm^2 impulse^{-1} and a net Na^+ entry of about 15.6 pmol.cm^2 impulse^{-1} were reported in electrically stimulated single frog muscle fibres (Hodgkin & Horowitz 1959). Others have calculated K^+ efflux of 7-11 nmol.g wet wt^{-1} contraction^{-1} for rat muscle (Creese et al. 1958; Clausen & Everts 1989; Everts & Clausen 1988). Balog & Fitts (1996) used ion-selective electrodes to study the effects of

fatiguing stimulation (150Hz) on $[Na^+]_i$ and $[K^+]_i$ in frog muscle. After the fatiguing stimulation $[Na^+]_i$ rose from 16mM to 49mM and $[K^+]_i$ fell from 142mM to 97mM. Correspondingly the membrane potential was depolarised from –83mV to –70mV after the fatiguing stimulation (Balog & Fitts 1996). Sjøgaard et al. (1985) studied the effects of fatiguing one-leg dynamic knee extensor exercise on intracellular and extracellular $[Na^+]$ and $[K^+]$ in humans. They measured the femoral a-v $[K^+]$ difference, estimated blood flow via the thermodilution technique and obtained muscle biopsies. Skeletal muscle extracellular fluid volume was determined from the inulin space. With an estimation of intracellular fluid volume the resultant ionic concentrations were determined. From these measurements it was calculated that $[Na^+]_i$ rose from 6 to 24 mM while $[K^+]_i$ dropped from 168 to 129 mM (Sjøgaard et al. 1985). There is a marked rise in plasma $[K^+]$ (from 4 to 7-8 mmol l^{-1}) during intense running or cycling exercise (Hermansen et al. 1984; Kowalchuk et al. 1988). The measurement of venous $[K^+]$ draining exercising muscle is likely to underestimate interstitial $[K^+]$ due to concentration gradients between the interstitium and blood as well as the dilution with blood arising from less active fibres (Juel et al. 2000b). The development of the microdialysis technique (Green et al. 1999b, Juel et al. 2000b) has enabled interstitial $[K^+]$ to be measured in human skeletal muscle. From resting values of 3.9-4.3mM, values of >9mM have been shown for interstitial $[K^+]$ during dynamic exercise at 50W (Juel et al. 2000b). These values are clearly higher than plasma $[K^+]$ values recorded during submaximal exercise (60% of max) of around 5.5mM (Vøllestad et al 1994).

2.5 Activation of the Na^+,K^+-ATPase enzyme

The Na^+, K^+-ATPase enzyme is rapidly activated upon exercise and this activation constrains the rise in $[Na^+]_i$ and $[K^+]_e$, limits membrane depolarisation and contributes to the maintenance of muscle excitability. The mechanisms for activation include a

combination of electrical, ionic and hormonal factors (Fig 2.3) and will be discussed in the following sections.

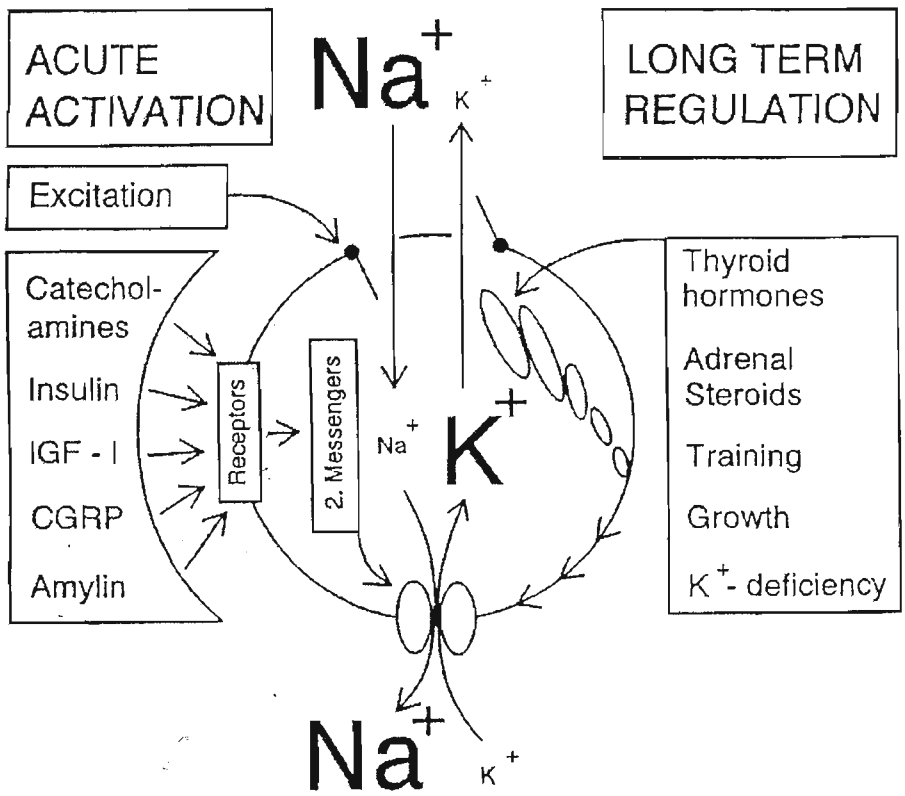

Figure 2.3 Regulatory factors controlling acute increases in activity/activation and long-term regulation influencing total content of Na^+, K^+-ATPase in skeletal muscle (From Clausen 1998).

2.5.1 Extent of Na^+, K^+-ATPase activation

At rest, the Na^+, K^+-ATPase enzyme activity is only a few percent of its theoretical maximum (Clausen et al. 1987; Clausen & Everts, 1989; Everts & Clausen 1994; Hazeyama & Sparks 1979). It has been well documented that excitation of muscle stimulates the Na^+, K^+-ATPase (Hazeyama & Sparks 1979; Juel 1986; Nielsen & Clausen 1997). The major factor underlying the rapid activation of the Na^+, K^+-ATPase during exercise is related to events associated with the action potential. The Na^+, K^+-ATPase

pump rate increases with increasing frequency of contraction (Clausen & Everts, 1989, 1994). Using supraphysiological stimulation frequencies (120 Hz) with an unloaded isotonic mounting, all available membrane Na^+, K^+-ATPase can be fully activated following 10 seconds stimulation in rat soleus muscle (Clausen & Nielsen 1994a, 1994b). At the other end of the spectrum, stimulation at only 2Hz can activate the Na^+, K^+-ATPase without changes in gross intracellular [Na^+] or extracellular [K^+] (Everts and Clausen 1994, Everts et al. 1988).

The physiological importance of these high stimulation frequencies has been questioned (Jones 1996). It is noteworthy that the motor unit discharge for standard bicycle ergometer exercise is around 25Hz (Sjøgaard et al. 1985). Frequencies in excess of 50Hz are rarely seen in human voluntary contraction (Jones 1996). This would theoretically only activate the Na^+, K^+-ATPase enzyme to half its maximal capacity suggesting that in human skeletal muscle, the available Na^+, K^+-ATPase are never fully activated. A possible explanation for incomplete Na^+, K^+-ATPase activation have alluded to cell depolarisation and muscle fatigue being a myoprotective mechanism (Green 1998). Others have suggested that the released [K^+] is an important stimulus to various physiological functions including vasodilation and ventilation (Kjellmer 1965; Linton & Band 1985; Murphy & Brayden 1995; Paterson 1996; Wildenthal et al. 1968).

2.5.2 Role of intracellular Na^+ in Na^+, K^+-ATPase activation

Hazeyama & Sparks (1979) showed that the Na^+, K^+-ATPase is activated by around 65% within the first 3 minutes of stimulation at 4 Hz. It has been suggested that this activation is the result of changes in intracellular Na^+ and extracellular K^+ (Juel, 1986). In mouse soleus muscle a 10.6mM increase in $[Na^+]_i$ resulting from 1 min electrical stimulation at 40Hz was followed by a rapid Na^+ extrusion in recovery (Juel 1986). Resting membrane

potential also recovered within 0.9 min (Juel 1986). Everts & Clausen (1994) later showed that a 60Hz tetanic stimulation for 10 sec induced a 58% rise in $[Na^+]_i$, which was followed by a ouabain suppressible rapid efflux of Na^+ corresponding to ~50% of the theoretical maximal transport capacity of available Na^+, K^+-ATPase. They reasoned that the activation of the Na^+, K^+-ATPase was linked to opening of sarcolemmal Na^+ channels, as Na^+, K^+-ATPase activation was blocked by tetrodotoxin, which is specifically blocks voltage sensitive Na^+ channels. Further, increased Na^+, K^+-ATPase activity occurred in the presence of veratridine, which maintains Na^+ channels in the open state (Everts and Clausen 1994). Lederer and colleagues (1990) introduced the notion of a subsarcolemmal "fuzzy space" which is defined by a functional compartment of the cytosol where diffusion of Na^+ out of the fuzzy space seems to be restricted compared to other parts of the cytosol. This could give rise to a rapid localised increase in $[Na^+]_i$ and could also contribute to Na^+, K^+-ATPase activation without an increase in total cellular $[Na^+]_i$ (Lederer et al. 1990; Semb & Sejersted, 1996).

Nielsen and Clausen (1997) demonstrated that mechanisms other than increases in intracellular $[Na^+]$ are also important in activating the Na^+, K^+-ATPase. They showed that the net extrusion of Na^+ following 30s stimulation of rat soleus muscle at 60Hz continued for up to 10 min reducing intracellular $[Na^+]$ to values 30% below resting levels.

2.5.3 Maximal Na^+, K^+-ATPase activation

As 3 Na^+ ions are pumped per ATP molecule split and each Na^+, K^+-ATPase molecule splits 8000 ATP molecules per minute at 37°C (Plesner & Plesner 1981) then a theoretical maximum Na^+ transport capacity of 16,800 nmol.g wet wt^{-1}. min^{-1} is derived. This is well above the maximum Na^+ and K^+ fluxes measured in rat soleus muscle

(Clausen & Kohn 1977), indicating that the Na^+, K^+-ATPase never reaches its theoretical maximal pumping rate *in vivo*. Clausen & Everts (1988) indicate that during maximal contractile activity, the net loss of K^+ (around 4.6nmol.g^{-1} wet wt. per rat soleus contraction, (Sreter, 1963)) from the working muscles is likely to exceed the capacity for reaccumulation via the Na^+, K^+ ATPase. Conversely, even a short recovery period is sufficient to reduce the previously elevated $[K^+]_e$ to near resting levels, illustrating the considerable functional capacity of the Na^+, K^+-ATPase (Clausen & Everts 1989).

2.5.4 Acute Hormonal activation

The Na^+, K^+-ATPase is regulated by a number of hormones, which act acutely by increasing the activity of existing Na^+, K^+-ATPase, eg. insulin, catecholamines and to a lesser extent, aldosterone (Clausen & Flatman 1977; Clausen & Hansen 1977). The mechanisms for rapid increases in Na^+, K^+ ATPase activity are brought about by variations in substrate concentrations, or by changes in intracellular signalling to initiate kinetic changes in the Na^+, K^+-ATPase. The Michaelis constant (K_m) of the Na^+, K^+-ATPase for ATP is 0.5-0.8mM (Sweeney & Klip 1998) which means that the ATP site on the Na^+, K^+-ATPase is likely to be saturated under most physiological conditions. Some of the proposed signalling cascades involved in the stimulation of Na^+, K^+-ATPase activity include 3', 5'-cyclic monophosphate (cAMP), protein kinase A, diacylglycerol activation of protein kinase C, and changes in intracellular free Ca^{2+} activation of calmodulin kinase (Ewart & Klip 1995). These signalling cascades generally result in phosphorylation of the Na^+, K^+-ATPase. Studies have demonstrated that direct phosphorylation of the Na^+, K^+-ATPase α subunit can result in modulation of its activity (Middleton et al. 1993). Acute hormonal regulation involves changes in Na^+, K^+-ATPase turnover number, affinity for substrates and or abundance at the sarcolemma through

translocation from intracellular storage areas. The stimulation of various hormones on Na^+ efflux (indicating Na^+, K^+-ATPase activity) is well summarised by Clausen (1996b). The extent of hormonal stimulation in comparison to a theoretical maximum for electrical stimulation for rat soleus muscle is however, small, as shown in Fig 2.4.

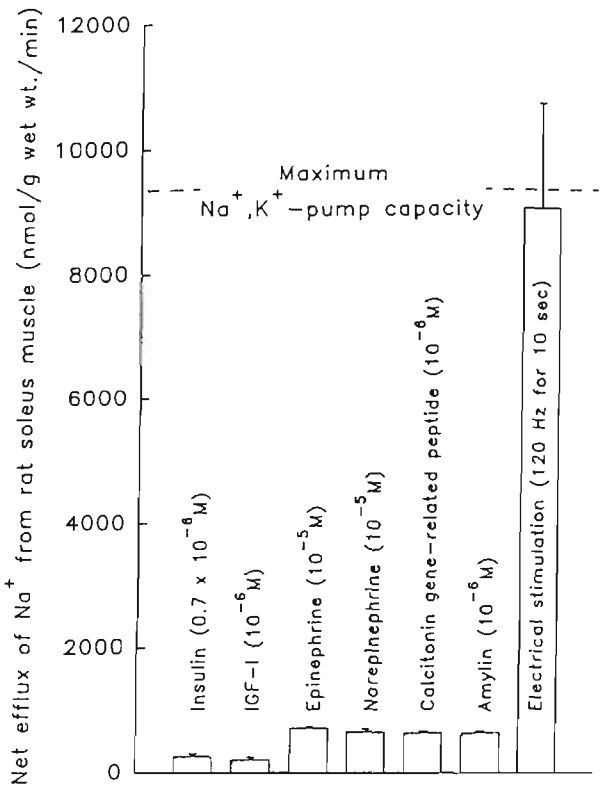

Figure 2.4 Rates of Na^+, K^+-ATPase activation via various stimuli. From Clausen (1996b).

2.5.4.1 Catecholamines

During exercise, increased sympathetic nervous system activity stimulates the release of the catecholamines, adrenaline and noradrenaline (Allsop et al. 1990; Kjær 1989; Mazzeo 1991). Adrenaline and noradrenaline also provide for the fine-tuning of Na^+, K^+-ATPase

enzyme activity in response to perturbations in the ionic environment. Catecholamines stimulate Na^+-K^+ transport via a β_2-adrenoreceptor-mediated stimulation of adenylate cyclase (Clausen & Flatman 1977, 1980; Wang & Clausen 1976). Adrenaline has been shown to stimulate skeletal muscle Na^+, K^+-ATPase *in vitro*, through second messengers (cAMP) and activation of protein kinase C (Clausen & Hansen 1977). Adrenaline and noradrenaline have been shown to stimulate K^+ uptake, as assessed by ouabain-suppressible $^{86}Rb^+$ uptake in rat soleus and extensor digitorum longus (EDL) muscles (Everts et al. 1988; Pfliegler et al. 1983). This effect is more pronounced in the fast twitch EDL than soleus muscle (Everts et al. 1988). Catecholamine-stimulated Na^+-K^+ transport leads to cell hyperpolarisation (8.5 mV) and a decreased intracellular Na^+ to K^+ ratio in rat soleus muscle (Ballanyi & Grafe 1988). In humans, intra-arterial injection of adrenaline was found to increase the positive arteriovenous difference for K^+ in the resting forearm, reflecting increased muscular K^+ uptake (DeLalande et al. 1961). The regulatory significance of the stimulating effect of catecholamines on Na^+ extrusion and K^+ reuptake via the Na^+, K^+-ATPase is illustrated by the observation that exercise-induced hyperkalemia is increased with β-adrenoreceptor blockade (Hallén et al. 1994; Williams et al. 1984). However, β_2-adrenergic stimulation of the Na^+, K^+-ATPase does not promote K^+ clearance during exercise (Rolett et al., 1990). Thus it appears that adrenaline is an important stimulator of Na^+, K^+-ATPase activity in resting muscle but it has been suggested (Hallén et al., 1996) that there are more acute Na^+, K^+-ATPase activators during exercise such as electrical stimulation or local rises in $[Na^+]_i$ (Semb & Sejersted, 1996).

Everts et al. (1988) noted that when 2 Hz electrical stimulation was added to adrenaline stimulation in rat soleus muscle, the effect on Na^+, K^+-ATPase activity was not additive. This indicates that electrical stimulation and adrenaline stimulate Na^+, K^+-ATPase

activity via common steps. The release of catecholamines is predominantly centrally controlled (Anderson & Clausen 1993; Clausen 1986) and it has been proposed that their effect on skeletal muscle is reduced during contractile activity (Everts et al. 1988; Katz et al. 1985). It was believed that local factors such as contractile activity are more important in stimulating the Na^+, K^+-ATPase, as an increased rate of K^+ loss from contracting muscles has been shown during the first minutes of exercise under β-adrenergic blockade (Gullestad et al. 1995; Hallén et al. 1994). Hallén (1996) suggested that this initial increased K^+ loss would be balanced by intracellular Na^+ gain which would compensate for the lack of catecholamine stimulation of the Na^+, K^+-ATPase during steady-state exercise. Catecholamines reduce the Na^+, K^+-ATPase lag at the start of exercise (Hallén 1996). This line of reasoning is supported by the fact that intracellular $[Na^+]$ decreases when cells are exposed *in vitro* to terbutaline, a selective β_2 agonist (Juel 1988).

2.5.4.2 Insulin

In-vitro studies have shown that the anabolic hormone insulin increases the ouabain suppressible uptake of K^+ and extrusion of Na^+ in skeletal muscle (Clausen & Kohn 1977; Clausen et al. 1993; Creese 1961; Erlij & Grinstein 1976). The mechanisms whereby insulin increases Na^+, K^+-ATPase activity depends on the target tissue, but is thought to involve elevation of $[Na^+]_i$ or increased Na^+, K^+-ATPase affinity for Na^+_i, and translocation of Na^+, K^+-ATPase subunits to the sarcolemma (Ewart & Klip 1995; Lavoie et al. 1996; Sweeney & Klip 1998). In isolated muscle preparations, insulin has been shown to stimulate the uptake of K^+ via the Na^+, K^+-ATPase enzyme (Clausen & Flatman 1987, Minaker & Rowe 1987) and increase Na^+ efflux from isolated rat soleus muscle by between 25 and 70% (Chinet & Clausen 1984; Creese 1968). Flatman and Clausen (1979) showed that $100mU.ml^{-1}$ of insulin increased ^{42}K Influx in rat soleus muscle by

20% compared to a control condition. It has also been suggested that a negative feedback exists between plasma [K^+] and insulin secretion, whereby hyperkalemia induces increased levels of insulin, which in turn augments K^+ reuptake into skeletal muscle (Clausen & Everts 1989). The insulin stimulation of Na^+, K^+-ATPase activity in muscle, fat, liver and kidney (Clausen, 1986; Ewart & Klip 1995) will counteract the intracellular gain in [Na^+] that occurs through insulin-induced amino acid and glucose uptake. Skeletal muscle is also a major storage site for dietary K^+, so activation of the Na^+, K^+-ATPase would remove this ion from the blood after a meal (Lavoie et al. 1996).

An increased plasma membrane Na^+, K^+-ATPase $\alpha_2\beta_1$ isoform expression with parallel decreases in expression of these isoforms in the isolated intracellular membrane fragment was shown after exposure of rat skeletal muscle to 30 min of 1.5 units of insulin (Hundal et al. 1992). Immuno electron microscopy has confirmed that insulin induces β_1 isoform redistribution (Lavoie et al. 1996).

2.5.5 Exercise-induced Na^+, K^+-ATPase isoform translocation

Two studies suggest that acute exercise can induce translocation of Na^+, K^+ ATPase isoforms to the plasma membrane (Tsakiridis et al. 1996, Juel et al. 2000a). Tsakiridis et al. (1996) showed that treadmill exercise of 1 hour duration (20m/min, 10% grade) increased the plasma membrane content of Na^+, K^+-ATPase α_1 (64%) and α_2 (43%) subunits in oxidative muscles of rats. However, they could not demonstrate the existence of a donor pool of pre-existing Na^+, K^+-ATPase α subunits, with no significant change in subunit content found in the isolated intracellular fractions. A significant increase in β_1 subunits at the plasma membrane was found while the β_2 subunit expression in the plasma membrane increased in type IIb fibres of the rat (Tsakiridis et al. 1996). The amount of Na^+, K^+-ATPase protein at the plasma membrane is dependent upon the rate of

arrival, retrieval and degradation. Therefore it is conceivable that the increased presence of $\alpha_1\beta_2$ at the plasma membrane is due to decreased retrieval and degradation.

The observations of the Tsakiridis study are in agreement with Joreteg & Jogestrand (1986) who demonstrated increased digoxin binding to thigh muscle in human subjects undergoing 1hr of cycle ergometer exercise. The subjects exercised at 140-180W after two weeks of treatment with digoxin. Digoxin binding to thigh skeletal muscle increased 20% while serum digoxin concomitantly decreased by 40% (Joreteg & Jogestrand 1986). The increased binding of digoxin to skeletal muscle with exercise could be due to increased insertion of appropriate $\alpha_1\beta_2$ dimers at the plasma membrane and/or an increased Na^+, K^+-ATPase activation promoting digoxin binding.

The study of possible isoform translocation in human skeletal muscle is limited given that 5-6g of tissue is usually required to assess Na^+, K^+-ATPase subunit translocation using membrane fractionation techniques. However, a recent study by Juel et al. (2000a) incorporated production of sarcolemmal giant vesicles (Juel 1991) to analyse the biopsied muscle. Juel et al. (2000a) showed that humans performing exhaustive knee extensor exercise (average 90W for 4.6 min) increased their sarcolemmal content of α_2 and β_1-subunit isoforms by 70% and 26%, respectively.

2.5.6 Other hormones

Some other hormones which are of lesser importance during exercise have been shown to stimulate Na^+, K^+-ATPase activity (Andersen & Clausen 1993; Dørup & Clausen 1995). These hormones which increase the active Na^+, K^+ transport rate include amylin, calcitonin-gene related peptide (CGRP) and insulin-like growth factor I (IGF-I) (Anderson & Clausen 1993; Dørup & Clausen 1995). CGRP is present in both motor nerves and afferent free nerve endings (Sakaguchi et al. 1991). It is postulated that

CGRP is released during nerve impulse activity (Sakaguchi et al. 1991; Sala et al. 1995) and that it's stimulatory action is catecholamine-like (Andersen & Clausen 1993). CGRP has been shown to increase ^{86}Rb uptake in rat soleus muscle by up to 77%, depending on age (Andersen & Clausen 1993). Amylin is colocalised in the pancreatic β-cells and is cosecreted with insulin where it can acutely increase Na^+, K^+ ATPase activity through increasing cAMP in the cell (Clausen 2000). In rat soleus muscle, amylin was found to induce a 45% stimulation of Na^+ efflux and a 43% increase in ^{86}Rb uptake (Clausen 2000). It has similar structural similarity to CGRP and exerts a similar effect on active Na^+ -K^+ transport (Clausen 1996b). IGF-I induces hypokalemia in normal human subjects (Giordano & DeFronzo 1995), which may be due to its stimulatory effect on K^+ uptake in skeletal muscle (Dørup & Clausen 1995). IGF-I has been shown to increase ^{86}Rb uptake by 54% in rat soleus muscle (Dørup & Clausen 1995). In regard to their capacity to activate the Na^+, K^+-ATPase enzyme, these other hormones also have a relatively low quantitative importance in comparison to electrical stimulation (Clausen 1996, Fig 2.4).

2.6 Skeletal muscle fatigue

2.6.1 Sites of Muscle fatigue

Exercise-induced muscle fatigue is defined as a transient loss of power generating capacity resulting from preceding physical activity (Asmussen 1979). Fatigue is a gradual process from the beginning of exercise, is an integral part of physical activity (Bigland-Ritchie et al. 1986a, Gandevia 1998), and is a complex phenomenon with multiple factors involved. The importance of each is dependent on intensity and duration of exercise, muscle fibre type, recruitment pattern, and individual degree of physical training (Fitts 1994). Fatigue is associated with reductions in maximal isometric force,

shortening velocity and a slowing of relaxation (Allen et al. 1995; de Haan et al. 1989). Some of these factors will be discussed in the following section, before mention is made of the importance of the Na^+, K^+-ATPase in the etiology of skeletal muscle fatigue. Both central and peripheral sites potentially involved in fatigue are discussed.

2.6.2 Central Fatigue

A combination of central and peripheral factors is proposed to induce the loss in force generating capacity in skeletal muscle (Gandevia 1998). Central fatigue is related to events of neural input to higher brain centres, recruitment of the alpha motor neurones, and the alpha motor nerves. The underlying mechanisms are not fully understood but Bigland -Ritchie et al. (1986b) showed that slowing of motor neuron firing rates during fatigue possibly resulted from feedback from muscle group III & IV afferents. Gandevia et al. (1992) demonstrated a progressive reduction in voluntary drive to motorneurones during isometric contractions, indicating central fatigue. However, James et al. (1995) found central fatigue to contribute to a relatively small effect (< 20%) to the total loss of power elicited by electrical stimulation in a human voluntary isokinetic contraction of knee extensors. Most research points to fatigue beyond the neuromuscular junction (Fitts 1994, Green 1998). The following sections therefore examine the factors contributing to peripheral muscular fatigue.

2.6.3 Peripheral fatigue

Research on peripheral factors in muscle fatigue have focussed on excitation-contraction coupling and metabolic energy supply. Each step in the activation process is a potential site for muscle fatigue. ATP is required at a number of sites including actin-myosin

crossbridge cycling (Myosin ATPase) as well as ion transport processes involving the Na^+, K^+-ATPase and Ca^{2+} -ATPase enzymes.

2.6.4 Actin-myosin interaction

A decline in muscle force development has been associated with increases in muscle H^+, lactate and P_i concentrations during high intensity exercise (Cady et al. 1989; Hogan et al. 1995; Wilson et al. 1988). A three to ten-fold increase in P*i* (Bigland-Ritchie et al. 1983) and a decline in muscle pH from around 7.0 to 6.4 (Hermansen & Osnes 1972; Metzger & Fitts 1987; Thompson et al. 1992) have been associated with fatigue. Godt and Nosek (1989) showed that a low pH impeded skinned rabbit muscle crossbridge interaction (by interfering with Ca^{2+} binding to troponin C) and was an important component of the decline in force. More recent studies have challenged these findings, showing that the depressive effect of reduced pH on force is dependent upon muscle temperature (Pate et al. 1995; Westerblad et al. 1997). In skinned rabbit psoas muscle, at a temperature of 10°C, lowering the pH induced a large force decline, but only a 10% decline in force was evident at a muscle temperature of 30°C (Pate et al., 1995). Thus acidosis had little effect on force production at physiological temperature (Westerblad et al. 1997). The mechanism is likely to be associated with reduced myofibrillar Ca^{2+} sensitivity (Godt & Nosek 1989; Lamb et al. 1992; Westerblad & Allen 1991). Low [ATP] and increases in H^+ and Pi also reduced myofibrillar ATPase activity *in vitro* (Parkhouse 1992).

2.6.5 Excitation-contraction coupling

Muscle excitation involves the spread of the action potential along the sarcolemma and down the t-tubules. Then the voltage sensor in the t-tubule responds to the depolarisation, inducing Ca^{2+} release from the sarcoplasmic reticulum (SR, Rios et al. 1991). The

dihydropyridine receptor is the t-tubule voltage sensor and the ryanodine receptor is the SR Ca^{2+} release channel (Rios et al. 1991). Impaired SR Ca^{2+} release and reuptake have been implicated in the fatigue process (Booth et al. 1997; Favero et al. 1999; Hargreaves et al. 1998; Westerblad et al. 1993). A reduction in tetanic intracellular [Ca^{2+}] during fatiguing contractions of single fibres (Westerblad & Allen 1996) suggests an impaired SR Ca^{2+} release and metabolic factors are important in this occurrence. Metabolic by-products such as P*i*, and Mg^{2+} inhibit Ca^{2+} release in isolated SR (Favero et al. 1995). However a rise in [H^+] does not inhibit SR Ca^{2+} release in intact skeletal muscle fibres (Lamb 1994). A rise in intracellular Mg^{2+} would also reflect a decline in [ATP]. A decline in [ATP] may also inhibit SR Ca^{2+} release as the SR release channel requires ATP (Smith et al. 1985). Impaired SR Ca^{2+} uptake rates and SR Ca^{2+} -ATPase activity have been observed with fatigue induced by both prolonged and short-term exhaustive exercise (Booth et al. 1997; Byrd et al. 1989a, 1989b; Li 1999). Local depletion of [ATP] near the SR Ca^{2+} -ATPase and a potential link with glycogen have been proposed (Chin & Allen 1997; Cuenda et al. 1993).

A decline in muscle SR Ca^{2+} ATPase activity with long duration exercise in humans has been demonstrated (Booth et al. 1997). As pH, temperature and energy supply are not limited in the Booth et al. (1997) *in vitro* assay, the decline in SR release and reuptake suggest a long lasting structural alteration in the SR release channel. This structural alteration could be due to reactive oxygen species (Davies et al. 1992), elevated muscle temperature (Inesi et al. 1973), exercise-induced calcium activated proteases, (Belcastro et al. 1993), or other alterations in the Ca^{2+} release channel (Favero 1999; Gilchrist et al. 1992). The structural alteration may also explain the long-lasting, slow frequency fatigue observed following exercise (Westerblad et al. 1990). Due to structural homology of the Na^+, K^+-ATPase and Ca^{2+} ATPase α subunits (Inesi et al. 1997), the factors that affect

Ca^{2+} ATPase, may induce similar alterations in Na^+, K^+-ATPase function (Blanco & Mercier 1998; Lutsenko & Kaplan 1993).

2.7 Role of Na^+, K^+-ATPase in skeletal muscle fatigue

2.7.1 Membrane potential

During exercise, there is a net efflux of K^+ from muscle, which occurs during both high intensity (Vøllestad et al. 1994) and long duration exercise (Sahlin & Broberg 1989). The resultant decline in intracellular $[K^+]$ and rise in extracellular $[K^+]$ has been hypothesised to induce a decline in sarcolemmal membrane potential (Sjøgaard et al. 1985) and impaired membrane activation. This depolarisation of the membrane may block action potential propagation especially in the t-tubules, due to both a reduced distribution volume for the released K^+, and a lower density of Na^+, K^+-ATPase (Venosa & Horowitz 1981). The important role of the Na^+, K^+-ATPase in minimising this rise in extracellular $[K^+]$ and possible AP block was shown by Juel (1988) who showed that activation of the Na^+, K^+-ATPase with the β_2-agonist terbutaline during electrical stimulation of mouse soleus muscle decreased fatigue. This was attribute to decreased membrane depolarisation, a reduced fall in $[K^+]_i$ and a smaller rise in $[Na^+]_e$ (Juel 1988).

2.7.2 K^+ gradients and fatigue

An extracellular $[K^+]$ in excess of 8-10mM has been shown to reduce excitability in skeletal muscle, due to membrane depolarisation and slow inactivation of voltage dependent Na^+ channels (Cairns et al. 1995; Juel 1988; Lännergran & Westerblad 1986; Ruff et al. 1988). The amplitude and propagation velocity of the action potential is reduced (Balog & Fitts 1996; Hodgkin & Horowitz 1959; Juel 1988) leading to a loss of

contractile force (Holmberg & Waldeck 1980). Whilst only a 5% loss of contractile force in mouse soleus muscle was seen when $[K^+]_e$ was elevated to 7.5mM (Juel 1988), a 40% loss in contractile force was seen when $[K^+]_e$ was elevated to 10mM (Juel 1988). When extracellular $[K^+]$ was raised to 12.5mM there was a 95% loss in contractile force in rat skeletal muscle (Clausen et al. 1993). Muscle interstitial $[K^+]$ assessed via microdialysis has been reported above 9mM during dynamic exercise at 50W (Juel et al. 2000b), which would impair action potential propagation along the sarcolemma and t-tubule.

The blockage of action potential propagation in the t-tubule would then prevent activation of Ca^{2+} release channels in the sarcoplasmic reticulum and therefore prevent subsequent activation of actin for crossbridge interaction. This is supported by a study done in isolated single mouse fibres stimulated at 50Hz (Westerblad et al. 1990). These authors showed a reduction in $[Ca^{2+}]$ in the central region of the fibre, suggesting impaired t-tubule conduction. Opposing this is the observation that intra-membrane charge movement (a measure of t-tubule voltage sensor activity) was found to be unaltered during fatigue, in spite of a decline in tetanic intracellular Ca^{2+} levels of ~ 1μM (Györke 1993).

2.7.3 Na^+ gradients and fatigue

A reduced $[Na^+]_e$ has also been shown to reduce muscle excitability and contribute to the decline in muscle force (Bezanilla et al. 1972; Bouclin et al. 1995; Venosa 1974). It is possible that t-tubule $[Na^+]$ may fall during contractile activity, exposing this region to action potential block. Bezanilla et al. (1972) showed a reduction in tension in single fibres from frog semitendinosus muscle, while Venosa (1974) showed that frog sartorius twitch tension was completely abolished when $[Na^+]_e$ was decreased to 40mM. A 30% reduction in force was shown when extracellular Na^+ was substituted with choline in

isolated rat soleus muscle (Overgaard et al. 1997). The reduced muscle excitability induced by lowering extracellular Na^+ is more than likely related to the decreased sarcolemmal Na^+ gradient and reduced AP amplitude (Nielsen & Overgaard 1996). The reduced AP amplitude would decrease or inactivate the t-tubular voltage sensor thus limiting SR Ca^{2+} and subsequent force generation (Balog & Fitts 1996).

2.7.4 Synergistic interaction of decreasing $[Na^+]_e$ and increasing $[K^+]_e$ with fatigue

An important finding is that the inhibitory effect of reduced $[Na^+]_e$ on force production is exacerbated by that of increased $[K^+]_e$ (Bouclin et al. 1995; Overgaard et al. 1997, 1999). In frog sartorius muscle an increase in $[K^+]_e$ from 3 to 7mM whilst reducing the $[Na^+]_e$ from 120mM to 100mM decreased the tetanic force by 31% (Bouclin et al. 1995). Reducing the $[Na^+]_e$ decreased the $[K^+]_e$ at which twitch force and tetanic force were decreased or abolished. Thus, the combined effects of decreasing $[Na^+]_e$ and increasing $[K^+]_e$ were greater than the sum of their individual effects (Bouclin et al. 1995; Overgaard et al. 1997). Overgaard and colleagues (1997) and Bouclin et al. (1995) went on to suggest that the effects on force of these altered ionic concentrations were not simply additive but synergistic in nature. The importance of the Na^+, K^+-ATPase enzyme in minimising the decrease in $[Na^+]_e$ and rise in $[K^+]_e$ will be discussed is the next section.

2.7.5 Role of Na^+, K^+-ATPase in maintenance of contractility

The importance of active Na^+, K^+ transport in maintaining muscle excitability and force generation was shown by stimulating the Na^+, K^+-ATPase with salbutamol (Overgaard et al. 1997, 1999). Overgaard et al. (1997) showed that the loss of force in intact soleus muscle due to lowered $[Na^+]_e$ and increased $[K^+]_e$ could be 79% recovered by stimulating the Na^+, K^+-ATPase with 10^{-5} M salbutamol. The recovery of force was completely

inhibited with 10^{-3} M ouabain (Overgaard et al. 1997). The importance of active Na^+, K^+ transport was also demonstrated by inducing Na^+, K^+-ATPase deficiency. In isolated rat soleus and extensor digitorum longus muscles, ouabain-induced a marked but reversible reduction in force development (Nielsen & Clausen 1996). Further, a reduction in Na^+, K^+-ATPase content of 54-69%, induced by K^+ deficiency, caused a proportional loss in force during 60-90Hz stimulation in rat soleus muscle (Nielsen & Clausen 1994, 1996). The inhibitory effect of 12.5mM $[K^+]_e$ on muscle contractility was 75% recovered when Na^+, K^+-ATPase was stimulated with a combination of the β_2 agonist salbutamol (10^{-5} mM) and by insulin (100mU ml^{-1}, Clausen et al. 1993).

Clausen (1998) has also proposed that contractile performance is dependent on the leak/pump ratio for Na^+. This implies that Na^+ channel opening and influx of Na^+ (leak) is an important consideration in addition to the amount of Na^+ extrusion (pump) via the Na^+, K^+-ATPase. This was highlighted when rat soleus and EDL muscle were compared. The EDL muscle had 25% more Na^+, K^+-ATPase content but a much lower fatigue resistance than soleus muscle. The fast-twitch EDL muscle possesses twice as many Na^+ channels than soleus and therefore has a much larger Na^+ influx per twitch (Everts & Clausen 1992; Hansen Bay & Strichartz 1980; Ruff 1996). Thus the ratio between Na^+ channels to Na^+, K^+-ATPase enzymes in EDL is almost twice that of the soleus, which can be related to an increase muscle K^+ loss and Na^+ gain and thus decreased endurance and rate of force recovery (Harrison et al. 1997).

It is possible that metabolic changes within contracting skeletal muscle, such as a decline in the local [ATP] or increases in metabolites (P_i, H^+, or ADP) could impair Na^+, K^+-ATPase activity (Körge & Campbell 1995). A local fall in [ATP] may partially inhibit Na^+, K^+-ATPase activity, as shown in cultured epithelial cells in the rabbit (Kuwahara et al. 1998). Kuwahara and colleagues (1998) showed that an episode of ATP depletion

initiated by metabolic inhibitors such as rotenone, caused a partial inhibition of Na^+, K^+-ATPase activity as measured by ^{86}Rb uptake. Some investigators have suggested that a physiological lowering of cellular [ATP] can alter the association of Na^+, K^+-ATPase with the cytoskeleton (Molitoris et al. 1992) and cause internalisation of Na^+, K^+-ATPase molecules (Mandel et al. 1994). An impaired Na^+, K^+-ATPase activity would contribute to excessive $[K^+]_e$ accumulation, membrane depolarisation and thus, muscle fatigue. Excessive K^+ efflux during fatigue has been partly attributed to the opening of ATP sensitive K^+ channels (Renaud et al. 1996), which are discussed in the following section.

2.7.6 ATP-sensitive K^+ channels

ATP-sensitive K^+ channels or $K^+_{(ATP)}$ channels are voltage insensitive K^+ channels that open in the absence of ATP and close when ATP binds on the intracellular side of the channel (Renaud et al. 1996; Spruce et al. 1985). $K^+_{(ATP)}$ channel opening increases K^+ efflux and decreases the duration of the action potential (Renaud et al. 1996). These channels are not activated by low ATP levels, with half maximal inhibition between 20-140 μM (Nichols & Lederer 1991; Spruce et al. 1987). Even during heavy muscular contractions, [ATP] have not been measured in the $\mu mol.L^{-1}$ range, suggesting that the $K^+_{(ATP)}$ channels are less likely to be open in unfatigued and in fatigued muscle. Despite this, $K^+_{(ATP)}$ channels are open in fatigued muscle and it has been shown that the channel is activated by intracellular metabolites that accumulate with fatigue such as H^+, ADP, and lactate (Davies 1990; Renaud et al. 1996; Spruce et al. 1987). The most likely activator appears to be the reduction in muscular pH during exercise (Davies et al. 1992; Standen et al. 1992).

Blocking $K^+_{(ATP)}$ channels with tolbutamide or glibenclamide (both $K^+_{(ATP)}$ channel blockers) prolonged the action potential, which was linked to $K^+_{(ATP)}$ channels directly

increasing K^+ efflux, and contributing to the reduction in force (Renaud et al. 1996; Standen 1992). Further, in intact single fibres from mouse skeletal muscle, glibenclamide attenuated the decline in tetanic force seen during fatigue (Duty & Allen 1995). Renaud et al. (1996) did not support this finding, showing that glibenclamide does not affect the rate of fatigue development, but does prolong the rate of force recovery.

2.7.7 Reactive Oxygen species

Na^+, K^+-ATPase enzyme activity is dependent upon an intact phospholipid bilayer and any modifications to the phospholipid moiety could modify the protein's function. Lipid peroxidation and protein oxidation due to the presence of reactive oxygen species (ROS) have been shown to disrupt membrane proteins including the Na^+, K^+-ATPase (Kim & Akera 1987; Mishra et al. 1989). ROS or oxygen free radicals are produced in resting and exercising muscle in both the mitochondria and cytosol (Reid 1998). Davies and co-workers (1982) detected a 3-4 fold increase in free radical signals (via measurement of electron spin resonance, ESR) in rat hindlimb muscle after a single bout of exhaustive exercise. Subsequent ESR studies have confirmed this exercise-induced increase (Jackson et al. 1985; Kumar et al. 1992; Radak et al. 1995; Venditti & DiMeo 1997). It has been shown that ROS (including superoxides, hydroperoxides, lipid peroxyl radicals) may attenuate function and enhance fatigue in contracting oxidative muscle (Barclay et al. 1991; Powers & Lennon 1999; Reid 1996).

In the myocardium, the Na^+, K^+-ATPase is susceptible to ROS-induced lipid peroxidation, which induced a 90% depression in Na^+, K^+-ATPase activity in canine cardiac microsomal fractions (Kramer et al. 1984). The exact mechanism where Na^+, K^+-ATPase activity is compromised is not clear, but any disruption to the lipid bilayer (peroxidation) is likely to affect Na^+, K^+-ATPase activity (Robinson & Flashner 1979).

Myocardium Na^+, K^+-ATPase is susceptible to ROS- induced lipid peroxidation under ischaemic conditions as evidenced by a 3-fold increase in malondialdehyde, resulting in a 16% reduction in Na^+,K^+-ATPase activity (Kim & Akera 1987). It has also been shown in pig coronary artery that the Na^+, K^+-ATPase hydrolytic activity and transport of Rb^+ are uncoupled upon exposure to peroxide or superoxide (Elmoselhi et al. 1994).

The effects of ROS on skeletal muscle Na^+, K^+-ATPase have not been extensively studied. Only one study, Clough (abstract, 1996) reports a 70% decline in Na^+, K^+-ATPase activity in rat rectus femorus exposed to the free radical generating system of t-butylhydroperoxide and horseradish peroxidase. This inhibition of Na^+, K^+-ATPase activity in skeletal muscle probably involves disruption of the lipid bilayer, but the precise mechanism remains unresolved. It has been suggested that ROS have a significant impact on Na^+, K^+-ATPase, especially at the site on the enzyme bearing a sulphydryl (SH) group (Boldyrev et al. 1997; Körge 1998). Recent research also points to a role of metal-catalysed oxidation of the Na^+, K^+-ATPase in oxidative stress and possibly the fatigue process (Goldshleger et al. 1998). The extent to which this contributes to skeletal muscle fatigue remains to be determined.

2.7.8 Metabolic energy supply

The various cellular ATPases are potential sites for fatigue during exercise. A possible cause may be local decreases in ATP supply especially in relation to Ca^{2+} release (Allen et al. 1995; Owen et al. 1996). A criticism of this hypothesis is that muscle ATP levels are usually only reduced by 30-50% following intense exercise (McCartney et al. 1986; Söderlund & Hultman 1991; Spriet et al. 1987). The effect of these changes in [ATP] are unlikely to affect ATPase function as the [ATP] always remains up to an order of magnitude higher than the K_m for ATP for any muscle ATPase (Körge & Campbell

1995). However ATPase activity measurements are performed on whole muscle homogenates, therefore this does not exclude the possibility that in certain regions within the muscle, or within particular fibre types, the local [ATP] is limiting (Körge & Campbell 1995). Near the cellular ATPases with high ATP usage, it seems likely that the [ATP] will drop considerably below the total level in the cytoplasm (Lamb 1998). The possible impairment of ATPase function may be a safety mechanism limiting irreversible damage in fatiguing exercise. The concept of intracellular compartmentalisation of ATP may also play an important role (Körge & Campbell 1995). It could be speculated that skeletal muscle may protect itself from the rigor resulting from ATP depletion, through compartmentalisation of ATP levels.

2.7.9 Intracellular [Ca^{2+}] in activation of Na^+, K^+-ATPase

Calcium ions are involved in the regulation of many processes in excitable cells (Breier et al. 1998; Racay et al. 1996; Sulová et al. 1998). Each action potential induces a pulse of Ca^{2+} release from the sarcoplasmic reticulum, causing a transient rise in intracellular [Ca^{2+}] (Rüegg, 1992). Whilst the Ca^{2+} transient actually decreases with fatigue, there is a progressive rise in the resting intracellular [Ca^{2+}] (Allen et al. 1995). In millimolar concentrations Ca^{2+} inhibits both the Na^+, K^+-ATPase hydrolytic activity (Huang & Askari 1982; Yingst et al. 1992) and transport activity of the enzyme (Stankovicová et al. 1995). With the aid of Ca^{2+} channel blockers such as verapamil it has been shown that Ca^{2+} exerts its effect on the Na^+, K^+-ATPase lipoprotein complex on the intracellular side of the sarcolemma (Stankovicová et al. 1995). It is possible that Ca^{2+} could exert its effect through calmodulin or calnactin as these Ca^{2+} binding proteins reduce the effective [Ca^{2+}] necessary for Na^+, K^+-ATPase inhibition from submillimolar to submicromolar levels (Sulova et al. 1998; Yingst et al. 1992). Gissel and Clausen (1999) showed that

chronic low frequency stimulation of rat muscle led to increased sarcolemmal Ca^{2+} uptake via Na^+ channels, and increased cytoplasmic [Ca^{2+}] has been attributed to various cellular damage pathways (Duan et al. 1990; McCutcheon et al. 1990). However the exact mechanism whereby raised intracellular [Ca^{2+}] affects the Na^+, K^+-ATPase remains unclear and warrants further investigation.

2.8 K^+ release during exercise

2.8.1 Plasma [K^+]

Intense exercise results in the movement of fluid from the vascular compartment to the interstitium and contracting muscle (Lindinger & Heigenhasuer 1991; Watson et al. 1993). This reduces the plasma volume and will concentrate plasma ions (McKenna 1995), thereby partly explaining the exercise hyperkalemia. More importantly, K^+ is released from the working muscle as a consequence of inadequate sarcolemmal Na^+, K^+-ATPase activity and a failure to restore the K^+ gradient across the sarcolemma during excitation (Lindinger & Sjøgaard 1991; Verburg et al. 1999). The site for measurement of extracellular K^+ can alter interpretation as it can be measured in venous and arterial blood (Medbø & Sejersted 1990; McKenna et al. 1997) and more recently in the interstitium (Figure 2.5, Green et al. 1999b; Juel et al. 2000b). Clearly the highest [K^+] values would be seen in the interstitium followed by venous blood draining the exercising muscle and [K^+] values in arterial blood would be lower due to mixture of venous blood from exercising and non-exercising areas. The factors involved in muscle K^+ release and reuptake and the sites for extracellular K^+ determination are shown in Fig 2.5.

Increasing the muscle mass recruited during exercise increases the arterial plasma [K^+] (McKenna 1995). During intense cycling exercise the widest arterio-femoral venous plasma [K^+] difference was 0.5 $mmol.l^{-1}$, and arterial plasma [K^+] peaked at 8 mM

(Vøllestad et al. 1994). A wider arterio-venous plasma [K^+] of 1 mmol.l^{-1} was shown during intense single knee extensor exercise but a lower peak arterial plasma [K^+] of 6 mM was attained (Juel et al. 1990). The lower arterial plasma [K^+] in single leg exercise compared to cycling has been attributed to a smaller muscle mass releasing less K^+ combined with a larger inactive muscle mass enabling greater K^+ clearance (McKenna 1995).

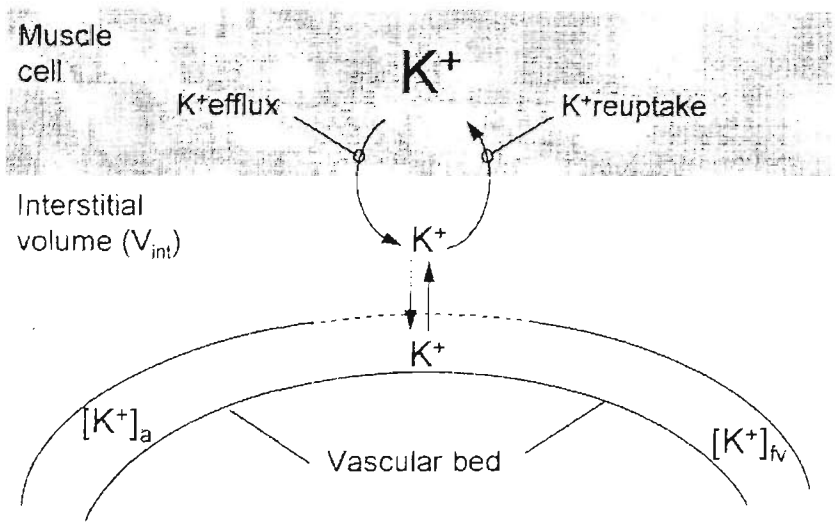

Figure 2.5 Schematic model of a muscle cell, interstitium and a capillary highlighting muscle K^+ efflux, reuptake and potential sites to measure extracellular K^+. (From Hallén 1996).

A linear rise in plasma [K^+], proportional to O_2 consumed, occurs during non steady state incremental exercise up to about 60% of $\dot{V}O_2$ max (Paterson et al. 1990). Thereafter there is an accelerated rise up to a maximum of around 6-7 mM in venous blood (Gullestad et al. 1989; Wilkerson et al. 1982). The curvilinear portion depends on the increasing power output and it has been suggested that the redistribution of K^+ at higher workrates is less efficient partly owing to decreased flow to non-exercising muscle (Vøllestad et al. 1994). High intensity treadmill exercise results in a pronounced increase

in arterial [K^+] from 3.5 to 8.3 mM in untrained human subjects (Medbø & Sejersted 1990).

Endurance training and sprint training reduces the arterial and venous plasma [K^+] during submaximal exercise at the same absolute workrate (Kiens & Saltin 1985; Kjeldsen et al. 1990a; Green et al. 1993, Table 2.2), and during all out sprint exercise (Harmer et al. 2000; McKenna et al. 1993, Table 2.2). This adaptation occurs rapidly with plasma [K^+] 26% lower during exercise after 6 days of training (Table 2.2, Green et al. 1993). The decreased hyperkalemia after training has partly been attributed to increased Na^+, K^+-ATPase content in trained muscle (see section 2.10.1 and Table 2.3).

Table 2.2 Training effects on plasma [K^+] during exercise in humans.

Study	n	Training duration and mode	Intensity	Δ plasma [K^+]
Kiens & Saltin 1985	5	8 wk constant load knee extension	70% pre-train maximum	40 % ↓ arterial [K^+]
Kjeldsen et al. 1990a	15	Endurance training, 10 wk military	Heavy submaximal	8 % ↓ arterialised-venous [K^+] in submaximal exercise
Green et al. 1993	9	Endurance training,, 6 days cycle 2h/day	60% pretrain $\dot{V}O_2$max	26 % ↓ Δ[K^+] during 60% pretrain $\dot{V}O_2$max
McKenna et al. 1993	9	sprint, 7 wk cycle 3/wk	maximal	27 % ↓ Δ[K^+] per unit work
Harmer et al. 2000		sprint, 7 wk cycle 3/wk	130% pretrain $\dot{V}O_2$peak	11 % ↓ peak plasma [K^+] 31% ↓ plasma Δ[K^+]

2.8.2 Physiological roles for K^+ release during exercise

The importance of K^+ release from skeletal muscle in fatigue has been documented, but plasma [K^+] also serves important physiological functions. These include hyperaemia,

the pressor response, arterial chemosensitivity and ventilation. Extracellular K^+ may exert an influence on sensory afferent fibres located in the interstitium. Thus increased $[K^+]_e$ may ensure optimal conditions for the exercising muscle as long as this is compatible with maintaining homeostasis for the rest of the body. Elevated $[K^+]_e$ facilitates muscle contraction through stimulation of local vasodilation, muscle reflexogenic input to the medullary cardiovascular and respiratory control centres, and excitation of arterial peripheral chemoreceptors (Paterson 1992, 1996; Sjøgaard 1990). It is possible that the time lag between muscle K^+ release and reuptake may not simply reflect inadequate uptake by the Na^+, K^+-ATPase (Hallén 1996; Verburg et al. 1999), but rather may be a well regulated mechanism to ensure a sufficiently high $[K^+]_e$ to mediate central and local adjustments to meet the demands of exercising muscle.

2.9 Alterations in Na^+, K^+-ATPase content

2.9.1 Factors influencing Na^+, K^+-ATPase content

The long-term regulation of Na^+, K^+-ATPase in skeletal muscle is achieved by variations in its sarcolemmal content through an increased synthesis/translocation and/or decreased degradation rate. Many factors alter Na^+, K^+-ATPase content in skeletal muscle, mainly due to chronic functional demand. These variations may occur as a physiological consequence of development and differentiation or alterations in physical activity with training, K^+ depletion, and a number of disease states (Clausen et al. 1982; Jebens et al. 1995; Kjeldsen et al. 1987; Schmidt et al. 1994). Thyroid hormone, chronic electrical stimulation, sprint and endurance training have been shown to increase Na^+, K^+-ATPase content (Azuma et al. 1993; Evertsen et al. 1997; Green et al. 1992b, 1993, 1999a; Hicks

et al. 1997; McKenna et al. 1993). The following sections review some of the factors inducing chronic alterations in Na^+, K^+-ATPase content.

2.9.1.1 Effect of age on Na^+, K^+-ATPase

Early development and differentiation of individual skeletal muscles is associated with a marked increase in Na^+, K^+-ATPase content (when expressed as pmol.muscle^{-1}) until maturity (adult form), when a plateau is reached (Kjeldsen et al. 1984a). Dauncey and Harrison (1996) showed that Na^+, K^+-ATPase content increases by 13% in guinea pig muscle through late gestation and then rapidly declines in the post-partum period (Table 2.3). However in rat skeletal muscle, from birth to four weeks, [^{3}H]-ouabain binding site content increased five-fold (Kjeldsen et al. 1984a), before decreasing by 58% from 4 to 12 weeks of age (Clausen et al. 1982). These values plateau to a mean content of around 250 pmol.g^{-1} wet weight at maturity (22 weeks of age) and remained relatively stable (22-86 weeks, Kjeldsen et al. 1984a). The explanation for the difference in postnatal changes between guinea pigs and rats probably lies in the fact that guinea pigs are born relatively more mature (Dauncey & Harrison 1996).

Few studies have been carried out on the early postnatal ontogeny of the Na^+, K^+-ATPase in humans. Kjeldsen and Grøn (1989) found no difference in Na^+, K^+-ATPase content from biopsies taken at autopsy from 2 month to 8 year old children. Most of these children died from sudden infant death syndrome (none had cancer and were not ill one week prior to death). Six older subjects (68yrs) tended to have a lower (14%) content of Na^+, K^+-ATPase than younger subjects (25yrs), but this did not reach significance (Klitgaard & Clausen 1989). Nørgaard (1984a) reported no age differences in a study of twenty healthy human subjects ranging from 25 to 80 years old.

In the rat hindlimb muscle, the α_1 isoform predominates through gestation and is not developmentally regulated, while mRNA for α_2 and α_3 don't appear until late gestation (Orlowski & Lingrel 1988). The abundance of β subunits increases during gestation. As the rat develops to the adult form, there is increased expression of the α_2 mRNA (Orlowski & Lingrel 1988). The β subunit mRNA increases significantly during the 14 days after birth but then declined to approx 50% after 55 days (Orlowski & Lingrel 1988).

Table 2.3 Effects of age on skeletal muscle Na^+, K^+-ATPase content

Study	Species	Muscle	n	Age	change in Na^+, K^+-ATPase content
Dauncey & Harrison 1996	guinea pig	soleus, EDL	nr	-late gestation -post partum	1350 at 85 days, 1550 at birth, ↓ post partum
Clausen et al. 1982	rat	soleus	nr	4-12 wks	58% ↓ from 4 to 12 wks
Kjeldsen et al. 1984a	rat	soleus	nr	0-4 wks 4-12 wks	5-fold ↑ from birth to 4 wks 69% ↓ from 4 to 12 wks
Kjeldsen & Grøn 1989	human (post mortum)	vastus lateralis	18	0-8 years	ns
Nørgaard et al. 1984a	human	vastus lateralis	20	25 vs 80 yrs	ns

ns: not significant; nr not reported

2.9.1.2 Effect of gender on Na^+, K^+-ATPase

The possibility of gender effects on Na^+, K^+-ATPase in humans is unclear. Nørgaard and co-workers (1984a) did not find a gender effect for Na^+, K^+-ATPase content in vastus lateralis biopsies from 20 healthy subjects. However a recent study showed highly

trained male cross country skiers had an 18% higher Na^+, K^+-ATPase content than highly trained females (Evertsen et al. 1997).

2.9.1.3 Effect of fibre type on Na^+, K^+-ATPase

In animal studies there is evidence that Na^+, K^+-ATPase and its subunits have a fibre type dependence (Clausen et al. 1982; Nørgaard 1986). Rat extensor digitorum long (EDL) muscle have a 20-30% higher Na^+, K^+-ATPase content than soleus (Clausen et al. 1982; Nørgaard 1986). More recently, in opposition to this a moderate positive relationship between Na^+, K^+-ATPase content and oxidative potential was reported in rat skeletal muscle (Chin & Green 1993). Dauncey and Harrison (1996) found no significant fibre type dependence in guinea pigs. Most studies on human skeletal muscle utilise biopsies from the vastus lateralis muscle which has a mixed fibre composition (Grimby et al. 1982; Nygaard 1981). However, there was no clear indication of a fibre type dependence in human skeletal muscle (Benders et al. 1992; Dørup et al. 1988; Madsen et al. 1994; Nørgaard 1986).

Northern blot analyses of rat skeletal muscle using isoform specific cDNA probes demonstrate that there is a fibre type specific isoform expression (Hundal et al. 1993). The α_1 and α_2 mRNA were present in both fast and slow twitch muscle with greater proportions in slow twitch muscle. The α_2 mRNA was four-fold more abundant than α_1 in slow twitch muscle. The β_1 mRNA and protein contents were 3-fold higher in slow twitch (soleus) muscle, while the β_2 mRNA isoform was 8-fold higher in fast twitch EDL muscle (Hundal et al. 1993). Protein expression of the β_1 subunit was predominantly in slow twitch muscle while fast twitch expressed mostly the β_2 subunit. Despite differences in mRNA, protein expression of the α_1, α_2 subunits were similar in both muscle fibre types. There are also potential differences in isoform translocation to the

plasma membrane between slow and fast fibres (Lavoie et al. 1996) as discussed in section 2.1.7. The above analyses were performed on isolated membrane fragments and this technique has been severely criticised due to poor recovery of enzyme activity (Hansen & Clausen 1996).

This fibre type specific isoform expression is of importance in human skeletal muscle due to its heterogenous muscle fibre type expression. The importance of possible human skeletal muscle Na^+, K^+-ATPase isoform specific function warrants investigation.

2.9.2 Chronic hormonal regulation of Na^+, K^+-ATPase

Changes in gene transcription and mRNA levels of the Na^+, K^+-ATPase subunits are often under the influence of regulatory hormones such as the thyroid hormones (Ewart & Klip 1995). There are a number of levels of long term hormonal regulation, from transcription to translation to stability of protein.

2.9.2.1 Thyroid hormone

Detailed reviews of the effect of thyroid hormone on Na^+, K^+-ATPase can be found elsewhere (Clausen 1986, 1991; Ewart & Klip 1995; Ismail-Beigi 1993). Briefly, thyroid hormones (TH, including T_3 and T_4) have been shown to increase the content of Na^+, K^+-ATPase molecules but not affect the specific activity of each enzyme (Ewart & Klip 1995; Kjeldsen et al. 1984c). Elevated TH in hyperthyroidism or injection of T_3 has been shown to increase Na^+, K^+-ATPase content in rat and human skeletal muscle (Azuma et al. 1993; Kjeldsen et al. 1984c; Lin & Akera 1978; Nørgaard et al. 1983). TH stimulates an increase in mRNA levels, leading to more sarcolemmal Na^+, K^+-ATPase, which are isoform- and tissue-specific. There is also evidence that TH regulates the Na^+, K^+-ATPase in an isoform specific manner. Azuma et al. (1993) showed that the $\alpha_2\beta_1$

heterodimer is most responsive to TH regulation, while the effect on α_1 and β_1 were tissue specific. As the Na^+, K^+-ATPase requires energy, it has been suggested that the increase in Na^+, K^+-ATPase content induced by TH could account for the thermogenic action, but this may only constitute around 15-20% of TH-induced thermogenesis (Biron et al. 1979; Clausen 1998; Ismail-Beigi & Edelman 1971).

2.9.2.2 Insulin

The chronic actions of insulin have been studied to a lesser extent compared to the short-acute effects of insulin on the Na^+, K^+-ATPase. This is most likely due to the fact that there are rarely states where insulin is elevated *in vivo*. However it is important to note that Na^+, K^+-ATPase is 48% decreased in hyperinsulinemic states such as diabetes (Kjeldsen et al. 1987). As well as insulin's acute effect in increasing Na^+, K^+-ATPase activity, it has also been linked to the movement of new Na^+, K^+-ATPase units to the sarcolemma (Lavoie et al. 1996; Marette et al. 1993). This increased Na^+, K^+-ATPase expression was shown in streptozotocin-induced diabetic rats with a 22% increase in Na^+, K^+-ATPase content after insulin administration (Schmidt et al. 1994). A 22% and 17% increase was also found in vastus lateralis muscle of humans with insulin-treated type I and type II diabetes respectively.

2.10 Chronic effects of increased physical activity on Na^+, K^+-ATPase

The following review focuses on skeletal muscle responses to chronic exercise, with particular attention to the Na^+, K^+-ATPase.

2.10.1 Effect of training on the Na^+, K^+-ATPase

2.10.1.1 Response of Na^+, K^+-ATPase to chronic electrical stimulation

Increased Na^+, K^+-ATPase expression has been shown in muscle exposed to chronic electrical stimulation (Green et al. 1992b; Hicks et al. 1997). In rabbit fast twitch EDL muscle, a 40% increased expression of Na^+, K^+-ATPase enzymes occurred after four days of chronic low frequency (10Hz) electrical stimulation (Green et al. 1992b). This increased further to an 86% rise compared to controls after only 10 days of stimulation (Green et al. 1992b). Hicks et al. (1997) investigated chronic low frequency (10 Hz) stimulation of rabbit fast-twitch muscle. They showed a 60% increase in Na^+, K^+-ATPase content after 6 days and a doubling in Na^+, K^+-ATPase content after 20 days electrical stimulation. This Na^+, K^+-ATPase increase from stimulation of fast twitch muscle preceded changes in myofibrillar proteins and together with a decrease in Ca^{2+}ATPase and ryanodine receptors, was suggested to be responsible for early functional alterations (Hicks et al. 1997). These functional alterations included increased fatigue resistance, absence of twitch potentiation and prolongation of the contraction and relaxation times. The increase in Na^+, K^+-ATPase content correlated with a recovery of the M-wave (Hicks et al. 1997), and this was further supported by Overgaard et al (1999) who showed a linear relationship between M-wave area and tetanic force. Overgaard et al (1999) also showed that M-wave area was recovered if muscles exposed to 9mM $[K^+]_e$, and 85mM $[Na^+]_e$ were treated with the β_2-adrenergic agonist, salbutamol which stimulated the Na^+, K^+-ATPase. These studies indicate that the Na^+, K^+-ATPase plays a vital role in the restoration of the action potential and fatigue resistance.

2.10.1.2 Na^+, K^+-ATPase content and exercise training.

Animal studies have shown that 6 weeks of swim training increased Na^+, K^+-ATPase content by 46% in rat hindlimb muscle (Kjeldsen et al. 1986), whilst 3 weeks of run training increased guinea pig muscle Na^+, K^+-ATPase content by 25% (Leivseth et al. 1995). Knochel et al. (1985) showed that 6 weeks of treadmill running increased dog gracilis muscle Na^+, K^+-ATPase activity by 165%. However these authors utilised extensive purification techniques which have been criticised due to poor membrane recovery (Kjeldsen et al. 1988). A number of studies have shown an increase in human skeletal muscle Na^+, K^+-ATPase content after physical training (see Table 2.4). Klitgaard & Clausen (1989) showed that 16 elderly men who were long term (>10yr) swim, run, or strength trained, had a higher content of ouabain binding sites (30, 32 and 45% respectively) compared to age-matched controls. Contrary to the increased Na^+, K^+-ATPase content found in elderly-trained subjects, a study on young military conscripts showed no increase in Na^+, K^+-ATPase content after 10 weeks of physical conditioning (Kjeldsen et al. 1990a). A possible explanation for the two differing results in humans is that the training activity of the military conscripts was not intense enough to challenge Na^+, K^+-ATPase synthesis. However a subsequent endurance training study showed that Na^+, K^+-ATPase content was increased by 13.6% after only one week of cycle ergometer training (Green et al. 1993). Two studies showed that previously trained runners and cross-country skiers could increase their Na^+, K^+-ATPase content by 15-16% as a result of increasing the intensity of training (Evertsen et al. 1997; Madsen et al. 1994). In the study by Evertsen et al. (1997) the effects of moderate or intense increases in training (5 months) were investigated in both male and female elite cross country skiers. Na^+, K^+-ATPase content increased 16% when the data was pooled, with no difference between the moderate or intense training groups, nor were there differences between gender in

response to intensified training. With the exception of Klitgaard & Clausen (1989) it is interesting that these training studies have reported similar relative increases in Na^+, K^+-ATPase content of around 13-16% (Evertsen et al. 1997; Green et al. 1993; Madsen et al. 1994; McKenna et al. 1993). However a recent study by Green and co-workers (1999a) found a 29% increase in Na^+, K^+-ATPase content after 11 weeks of endurance training, with most of the rise occurring in the first 3 weeks (22% increase). Green and co-workers (1999a) also found that 12 weeks of high intensity resistance training induced a 17% rise in Na^+, K^+-ATPase content.

2.10.1.3 Na^+, K^+-ATPase, circulating [K^+] and performance

Cross-sectional studies have shown that endurance training results in a blunting of the exercise-induced rise in plasma [K^+] (McCoy & Hargreaves 1992; Medbø & Sejersted 1994; Tibes et al. 1974). Endurance training studies have also shown blunted hyperkalemia when exercise is conducted at the same HR (Kjeldsen et al. 1990) or workrate (Green et al. 1993) after training. This reduced hyperkalemia during exercise has partly been attributed to an increased content of Na^+, K^+-ATPase in skeletal muscle (Green et al. 1993; McKenna et al. 1995). McKenna et al. (1993) showed that seven weeks of sprint training increased Na^+, K^+-ATPase content by 16%, but that this did not correlate with sprint performance. However, McKenna et al. (1993) did show that the delta (Δ) $K^+.work^{-1}$ ratio during repeated 30 sec sprints was 27% lower after sprint training. There was no correlation with time to fatigue when performing submaximal contractions at 60% of max isometric force isometric in knee extensor exercise (Klitgaard & Clausen 1989), but there was a weak correlation between Na^+, K^+-ATPase content and maximal isometric force. Green et al. (1999a) found that $\dot{V}O_2$ max correlated with Na^+, K^+-ATPase content. This was supported by the correlation between Na^+, K^+-ATPase

content and $\dot{V}O_2$ max, performance during a treadmill run, and the rank of the subject's performance as a cross country skier (Evertsen et al. 1997). However these authors cautioned that despite these correlations the content of Na^+, K^+-ATPase was a poor predictor of performance. Similarly, the 15% rise in Na^+, K^+-ATPase content in previously trained subjects showed no correlation with $\dot{V}O_2$ max or time to exhaustion (Madsen et al. 1994).

It is unclear whether the stress of training induces increases in Na^+, K^+-ATPase content via circulating endocrine factors or via a general stress response in untrained skeletal muscle, such as the arms of runners or cyclists (Lindinger & Sjøgaard 1991). If it does, then the blunting of exercise-induced hyperkalemia would more likely be due to increased uptake of K^+ by both active and inactive tissues, rather than just by trained muscle groups. No increase in Na^+, K^+-ATPase content was found in the diaphragm of rats with endurance training, in contrast to an increase of up to 46% in hindlimb muscles (Kjeldsen et al. 1986). However the diaphragm already has a 19% greater Na^+, K^+-ATPase content in the untrained state compared to hindlimb gastrocnemius muscle (Kjeldsen et al. 1986). This increased Na^+, K^+-ATPase content in the diaphragm may be linked to its role of repeated contractions and requirement for fatigue resistance.

Other factors that are likely to explain the reduced hyperkalemia seen after training might be a reduced muscle K^+ release via attenuated K^+ channel opening, reduced muscle blood flow, diluted K^+ in an expanded plasma volume (McKenna et al. 1995).

Table 2.4 Training effects on human vastus lateralis muscle Na^+, K^+-ATPase content and muscle function.

Study	n	age	Training	Results: Δ [^{3}H]-ouabain binding sites	Functional Adaptations and Exercise Improvement
1	16	68	comparison trained vs untrained	Trained 30-40% > cf untrained	linear relation b\w max isometric strength and [Na^+, K^+-ATPase]
2	15	20	ET, 10 wk	no Δ	blunted exercise-induced hyperkalemia after training, 7% ↑ in running distance
3	9	19	sprint, 7 wk cycle 3/wk	16% ↑	27 % ↓ [K^+] per unit work 13% ↑ work, fatigue index 6% ↓
4	9	20	ET, 6 days cycle 2h/day	14% ↑	blunted rise in plasma [K^+] during & following exercise, 7% ↑ in VO_2 max
5	39	30	↑ intensity of ET	15% ↑	6% ↑ in VO_2 max, 74% ↑ in endurance time
6	11 (male) 9(female)	18	↑ intensity of ET, 5 mo	16% ↑	[Na^+, K^+-ATPase] correlated with VO_2 max & 20 min run time
7	7	21	11 wk prolonged ET	29% ↑	[Na^+, K^+-ATPase] correlated with VO_2 max in prolonged ET
	9	20	11 wk high intensity RT	17% ↑	

1. Klitgaard & Clausen 1989; 2. Kjeldsen et al. 1990a, 1990b; 3. McKenna et al. 1993; 4. Green et al. 1993, 5. Madsen et al. 1994; 6. Evertsen et al. 1997; 7. Green et al. 1999a. ET, Endurance training; RT, Resistance training

2.10.2 Other effects of Training on Skeletal Muscle

The degree to which skeletal muscle responds to training varies due to a number of factors including the type of training and duration stimulus, the species being studied, muscle fibre composition, and individual variation. The responses to acute or chronic exercise differ, but the functional outcomes of these changes are to minimise the

disturbance to homeostasis or to reduce the impact of fatigue on skeletal muscle function (Holloszy & Coyle 1984).

2.10.2.1 Endurance training, muscle morphology and metabolic adaptations

Endurance training results in an improved ability to sustain submaximal contractions and to resist fatigue (Coyle 1999; Green et al. 1992a; Ingjer 1979; Saltin & Golnick 1983). Numerous morphological, metabolic and ionic changes are induced in skeletal muscle. Prolonged endurance training increases the proportion of oxidative fibres as determined by histochemical staining or through identification of molecular alterations in the myosin heavy chain isoform expression (Baumann et al. 1987; Green et al. 1979; Howald 1982). These changes include an elevation of type I and or IIa fibre proportions, and a decrease in type IIb fibre proportions (Fitts 1996; Green et al. 1979). The differentiation of Na^+, K^+-ATPase content between fibre types is pronounced in animals but is not evident in human skeletal muscle (Benders et al. 1992; Dørup et al. 1988; Madsen et al. 1994; Nørgaard 1986). Therefore the effects of fibre type changes may be of lesser importance to Na^+, K^+-ATPase in human that in rat skeletal muscle.

The metabolic adaptations to endurance training have been reviewed by Coyle (1999). Briefly, these marked metabolic adaptations to endurance training enhance the capacity for aerobic metabolism (Gollnick et al. 1973; Green et al. 1992a; Taylor & Bachman 1999). Endurance training increases muscle capillary density, mitochondrial number and volume and the maximal activity of oxidative enzymes (Andersen 1975; Andersen & Henriksson 1977; Gollnick et al. 1973; Holloszy & Coyle 1984).

2.10.2.2 Resistance training

An increased force generating capacity occurs as a result of resistance training, due to initial neural adaptations and subsequent muscle hypertrophy (Moritani and De Vries 1979; Volek et al. 1999). The hypertrophy or increase in muscle cross sectional area results from increased contractile protein and occurs in both type I and type II fibres, but is more pronounced in type II fibres (Hather et al. 1991; Kraemer et al. 1995; MacDougall et al. 1980;, Staron et al. 1990; Volek et al. 1999). With resistance training there are no fibre type transitions between the two major fibre pools (Luthi et al. 1986; MacDougall et al. 1980; Thorstensson et al. 1976), rather a IIb→IIa transition has been shown (Adams et al. 1993; Kraemer et al. 1995). There is a close relationship between expression of type I, IIa and IIb myosin heavy chains (MHC) and myosin ATPase activity (Pette & Staron 1997). A 19 week resistance training study of human subjects showed that specific isoforms of myosin ATPase changed where the IIb myosin heavy chain (MHC) composition decreased from 19% to 7% and IIa MHC increased from 48% to 60% (Adams et al. 1993). This fibre type change confers an increased oxidative capacity in the fast twitch fibres (Staron et al. 1991). Little change in creatine kinase, myokinase, or glycolytic (eg phosphofructokinase and lactate dehydrogenase) enzyme activities have been found with resistance training (Thorstensson et al. 1976; Tesch et al. 1987, Tesch 1992).

2.11 Muscle disuse

2.11.1 Effects of disuse on intracellular electrolyte balance

Despite the vital role of Na^+ and K^+ in skeletal muscle function, the effects of disuse on muscle electrolyte regulation during contractile activity remain relatively unknown.

Wroblewski and Edström (1983) showed that following tenotomy in rat soleus muscle, $[Na^+]_i$ rose by ~230% compared to controls, whilst intracellular $[K^+]$ fell by ~28% from control levels. However, Jebens et al. (1995) showed K^+ content (millimoles.kg^{-1} fat free dry mass) of the sheep vastus lateralis was increased by 40% after 9 weeks immobilisation. It is possible that immobilisation and length of disuse could explain these different results.

Although denervation differs from immobilisation, the detrimental effects on electrolyte regulation are also evident. Leader et al. (1984) showed that 3 days denervation increased EDL $[Na^+]_i$ by 57%, but $[K^+]_i$ was unaffected. Clausen et al. (1982) showed that denervation for 1 wk increased rat soleus $[Na^+]_i$ by 38% and decreased $[K^+]_i$ by 4%. Thus muscular disuse/denervation profoundly effects resting muscle electrolyte contents, and these changes may be due to either muscle membrane permeability changes and/or changes in the content of Na^+, K^+-ATPase. No studies have investigated electrolyte changes in human skeletal muscle with regard to disuse at rest or during contractile activity.

2.11.2 Effects of muscle disuse on Na^+, K^+-ATPase content

The decrease in metabolic and oxidative muscle fibre characteristics seen with muscle disuse are also evident in Na^+, K^+-ATPase content. These effects are summarised in Table 2.5. Inactivity induced by limb immobilisation causes a 20% reduction in the [^{3}H]-ouabain binding site content in rat muscle (Kjeldsen et al. 1986). Detraining after six weeks of endurance training caused a decrease in Na^+, K^+-ATPase content to pre-training levels (Kjeldsen et al. 1986). Leivseth and colleagues (1992) showed that the [^{3}H]-ouabain binding site content was decreased by 34% in guinea pig gastrocnemius muscle following 3 weeks of limb partial immobilisation. A more recent study by Jebens et al.

(1995) found that 9 weeks immobilisation in sheep vastus lateralis induced a 39% downregulation of Na^+, K^+-ATPase in the immobilised limb. Further, the contralateral limb demonstrated a 22% reduction in Na^+, K^+-ATPase content.

The adaptability of the Na^+, K^+-ATPase to functional demand was best demonstrated when a previously immobilised limb was trained or remobilised (Jebens et al. 1995; Kjeldsen et al. 1982; Leivseth et al. 1992). After run training, Na^+, K^+-ATPase content increased 93% compared to the 2 week immobilised condition and a 39% increase was seen in the control (not previously immobilised) gastrocnemius (Leivseth et al. 1992). A 38% increase was seen in the remobilised sheep vastus lateralis (Jebens et al. 1995). The 93% difference in Na^+, K^+-ATPase content for trained control muscles compares favourably with the 83% difference seen between swim-trained and plaster immobilised rat soleus muscle (Kjeldsen et al. 1986). While a few animal immobilisation and muscle disuse models have been investigated, only one muscle disuse study exists in humans (Leivseth et al. 1994). This study showed Na^+, K^+-ATPase content to decrease by 27% in the deltoid muscle of patients with shoulder impingement. Other studies have looked at patient groups (with their associated muscle disuse) and investigated Na^+, K^+-ATPase enzyme changes or ion regulation (Barlow et al. 1999; Hall et al. 1994; Nørgaard et al. 1990). This will be discussed in the next section with particular attention to lung transplant patients.

Table 2.5 Effects of reduced physical activity on skeletal muscle Na^+, K^+-ATPase content and function.

Study	Species	n	muscle	Intervention/ duration	Δ [^{3}H]-ouabain binding sites
Kjeldsen et al. 1986	rat	n.r	soleus	hindlimb tenotomy, 1 wk immobilisation, 1 wk	22% ↓ 20% ↓
Kjeldsen et al. 1986	rat	n.r	soleus, EDL	3 wks detraining after 6 wks endurance training	↓ to near pre-trained levels
Leivseth et al. 1992	guinea pig	7	gastroc-nemius	3 wks immobilisation, then 3 wks run training	19% ↓, then 57% ↑ with training
Leivseth and Reikerås. 1994	human	6	deltoid	Patients with shoulder impingement	27% ↓
Jebens et al. 1995	sheep	13	vastus lateralis	9 wks immobilisation	39% ↓

n.r not reported

2.11.3 Other morphological, metabolic and functional changes with immobilisation

In contrast to the vast literature on muscle adaptations seen with physical training, the morphological, metabolic, and functional changes in muscle with disuse have received relatively little attention. These are reviewed below.

2.11.3.1 Atrophy

Muscular disuse leads to a pronounced atrophy of muscle fibres and impairment of muscular function (Gibson et al. 1987). A common way to induce muscle disuse is immobilization, and these studies report muscular atrophy, evidenced by a decline in lean thigh volume and thigh cross sectional area, as well as a decline in muscle force (Halkjær-Kristensen & Ingemann-Hansen 1985; MacDougall et al. 1980). The decrement

in muscle mass has been attributed to both decreased protein synthesis within 6hr (Booth & Seider 1979) and increased protein degradation (Gibson et al. 1987; Goldspink 1977).

2.11.3.2 Fibre type alterations

A common finding in immobilisation studies with humans is a decrease in the proportion of type I fibres in quadriceps muscle (Halkjær-Kristensen & Ingemann-Hansen 1985), and/or atrophy of these fibres (Gibson et al. 1987, Häggmark & Eriksson 1981). These changes are summarised in Table 2.6. The proportion of type IIb fibres was increased in the soleus muscle after 6 weeks immobilisation (Häggmark & Eriksson 1979). In animal models, the suggested fibre transition during weightlessness is for a type I →type IIa →type IIb conversion (Martin et al. 1988; Pette & Staron 1997). Berg et al. (1997) does not support this in humans, with the percentages of type I, IIa, and IIb fibres, and the relative proportions of myosin heavy chain isoforms in vastus lateralis muscle, being unchanged after 6 weeks of bed rest. However a substantial atrophy in both type I and type II fibres has previously been shown (Hakkinen et al. 1985; MacDougall et al. 1980; Leivseth et al. 1987; Sargeant et al. 1977).

2.11.3.3 Metabolic changes

Only a few studies have investigated the effects of human skeletal muscle disuse on metabolic properties and these are summarised in Table 2.6. Immobilisation reduced muscle creatine phosphate and glycogen contents in human muscle (MacDougall et al. 1977). Activity of succinate dehydrogenase and cytochrome c oxidase as well as levels of GLUT4 protein concentration were reduced with 5-6 weeks of quadriceps immobilisation, while phosphofructokinase activity was unaffected (Halkjær-Kristensen & Ingemann-Hansen 1985; Häggmark et al. 1981; Blakemore et al. 1996).

2.11.3.4 Loss of muscle function

The functional consequences of the effects of disuse were investigated by measurement of muscular dynamic endurance in young soccer players (Halkjær-Kristensen & Ingemann-Hansen 1985). They showed a 27% decline in 6 minute maximal cycling performance in a group undergoing surgery followed by immobilisation, but only a 9% decline in those undergoing immobilisation. Sargeant et al. (1977) also reported a 15% decline in maximal O_2 consumption achieved during maximal one-legged cycling, after prolonged immobilisation. Healthy subjects undergoing 6 weeks bed rest showed a 25% decrease in maximum isometric knee-extensor torque (Berg et al. 1997). The combination of surgical trauma and subsequent muscle disuse also impair human isometric and dynamic muscle function (Sargeant et al. 1977).

Table 2.6 Effects of immobilisation on human vastus lateralis muscle morphology and metabolic and performance changes.

Study	Subject	n	Duration of Immob-isation	ΔC.S.A	Δ fibre proportion	Metabolic Δ	performance Δ
1	Unilateral leg fracture	7	4.5 mo	46%↓ type I 37% ↓ type II 12% ↓ leg vol.			17% ↓ in max O_2 uptake
2	patients	9	5 wks	26% ↓ type I		21% ↓ SDH no Δ in PFK	
3	Injured soccer players	84	4-6 wks	37% ↓ type I	12% ↓ type I no Δ type II	35% ↓ SDH no Δ in PFK activity	35% ↓ isokinetic peak torque
4	Tibial fracture patients	6	5 wks	14% ↓ type I	no Δ	↓ muscle protein synthesis	
5	Healthy subjects (T.B)	9	5 wks	6% ↓ upper arm girth		no Δ in ATP, ↓ CP 40%, ↓ glycogen 25%	35% ↓ max strength
6	Tibial fracture patients	6	6 wks	29% ↓ type I 26% ↓ type II		↓ cytochrome c oxidase, 50% ↓ GLUT4	
7	Healthy subjects	7	6 wk (bedrest)	19% ↓ type I	no Δ		25% ↓ max isometric torque

1. Sargeant et al. 1977; 2. Häggmark et al. 1981; 3. Halkjær-Kristensen & Ingemann-Hansen 1985; 4. Gibson et al. 1987; 5. MacDougall et al. 1987; 6. Blakemore et al. 1996; 7. Berg et al. 1997.

C.S.A: cross sectional area; T.B Triceps Brachii; SDH: succinate dehydrogenase; PFK: phosphofructokinase, Δ changes in.

2.11.4 Na^+, K^+-ATPase and transplant patients

Muscle disuse and chronic inactivity lead to reduced altered muscle morphology (see table 2.6), and lower Na^+, K^+-ATPase content (see table 2.5). Altered Na^+, K^+-ATPase function with disuse was postulated to limit exercise capacity in transplant patients (Hall et al. 1994). In Hall and colleagues' study (1994), heart and lung transplant patients displayed impaired plasma K^+ regulation, with a higher $\Delta[K^+].work^{-1}$ ratio compared to matched controls. Nørgaard et al. (1990) showed that patients with congestive heart failure had 25% lower skeletal muscle Na^+, K^+-ATPase content, and Barlow et al. (1994) also showed that such patients had higher arterial $[K^+]$ at similar low workloads to controls. Barlow and colleagues (1999) later measured Na^+, K^+-ATPase content and $\Delta[K^+].work^{-1}$ in CHF patients, but unfortunately did not compare these to healthy controls. However, Na^+, K^+-ATPase content was inversely correlated with $\Delta[K^+].work^{-1}$ before and after treatment of these patients with balloon mitral valvotomy. Three months after treatment with balloon mitral valvotomy, Na^+, K^+-ATPase content increased 14% and $\Delta[K^+].work^{-1}$ was reduced 36%.

2.11.5 Thoracic transplant patients and muscle disuse

For a considerable period of time before transplantation, lung transplant patients are usually chronically inactive and thus serve as an interesting model for muscular disuse. Lung transplantation has been shown to be an effective form of treatment for many forms of end-stage pulmonary disease, and allows most patients to return to near normal spirometry with only mildly impaired diffusion capacity (Williams et al. 1992). The exercise capacity of the transplant recipient increases post-operation (Pope et al. 1980; Theodore et al. 1987), but still remains only 50-60% of that seen in normal subjects

(Williams et al. 1992). Transplant patients also exhibit an earlier rise in blood lactate in incremental exercise tests (Kavanagh et al. 1988; Otulana et al. 1992; Ross et al. 1993; Theodore et al. 1987). The transplant appears to be successful in removing the ventilatory limitation to exercise (Williams et al. 1992), so other reasons for this exercise limitation post-transplant include poor motivation, low haemoglobin levels, impact of immunosuppressive medication and peripheral muscle defects (Gibbons et al. 1991; Otulana et al. 1992; Ross et al. 1993; Theodore et al. 1987). The poor exercise performance of patients post-thoracic lung transplant has been attributed to anaemia and skeletal muscle deconditioning (Banner et al. 1989; Hall et al. 1994; Otulana et al. 1992; Rudas et al. 1992; Savin et al. 1990; Theodore et al. 1987). However, Evans et al. (1997) have shown that neither ventilation, arterial O_2 saturation or mild anaemia could account for the decrease in aerobic capacity seen post lung transplantation. They showed that persistent abnormalities in skeletal muscle oxidative capacity with a resultant increased reliance on glycolytic energy supply was related to this low $\dot{V}O_2$ peak. Hall and colleagues (1994) suggested that peripheral muscle deconditioning contributed to the poor exercise performance post-thoracic lung transplantation, given that neither cardiac nor ventilatory function could account for the poor exercise performance. For example Williams and colleagues (1992) noted that although single and double lung transplant recipients had reduced $\dot{V}O_2max$ (46-50% of predicted) post-transplantation, the limitation did not appear to be ventilatory in nature.

2.12 Aims and Hypotheses

2.12.1 General Aims

This thesis examines the effect of acute exercise, chronic training and chronic disuse associated with lung transplantation on human skeletal muscle Na^+, K^+-ATPase and plasma [K^+] responses during exercise. Techniques used to assess Na^+, K^+-ATPase activity and content were maximal K^+-stimulated 3-*O*-MFPase activity in crude muscle homogenates and [^{3}H]-ouabain binding site content, respectively. The first study (Chapter 4) involved the modification of a sensitive technique for measurement of Na^+, K^+-ATPase activity in human skeletal muscle. The maximal K^+ stimulated 3-*O*-MFPase was modified from previous techniques (Benders et al. 1992; Norgaard et al. 1984b) so that Na^+, K^+-ATPase activity could be reliably measured in human skeletal muscle.

There is a well known depressive effect of acute exercise on skeletal muscle SR Ca^{2+} ATPase activity (Booth et al. 1997) but no studies have looked at the effects of an acute bout of fatiguing exercise on Na^+, K^+-ATPase activity. The second study (Chapter 5) therefore investigated 3-*O*-MFPase activity in muscle biopsies taken from untrained males at rest and immediately after 50 maximal knee extensor contractions.

The third study (Chapter 6) investigated the effects of training on Na^+, K^+-ATPase activity and content utilising endurance trained and resistance trained subjects. This study aimed to determine whether endurance trained and resistance trained muscle protected against any depressive effects of fatigue on Na^+, K^+-ATPase activity. The rationale for this study was that although endurance training (Green et al. 1993), and recently resistance training (Green et al. 1999a) have shown an increase in Na^+, K^+-ATPase content, no studies had investigated changes in Na^+, K^+-ATPase activity and content with training. A cross-sectional study design was chosen to maximise likely differences in

Na^+, K^+-ATPase content (Klitgaard & Clausen 1989) while the relationship between Na^+, K^+-ATPase and plasma [K^+] response during exercise was also explored.

The fourth study (Chapter 7) involved the other end of the physical activity spectrum, examining the effects of chronic muscle disuse associated with lung transplant patients. The study investigated skeletal muscle Na^+, K^+-ATPase activity and content as well as plasma [K^+] during exercise in these patients. Lung transplantation had removed the ventilatory limitation to exercise, so that the effects of other factors including chronic muscle disuse on Na^+, K^+-ATPase function in skeletal muscle could be evaluated.

2.12.2 Hypotheses

The hypotheses tested in this thesis were:

1. Na^+, K^+-ATPase activity assessed by maximal *in vitro* 3-*O*-MFPase activity (Chapter 4) in vastus lateralis muscle from healthy untrained individuals will be depressed by intense fatiguing knee extensor exercise (Chapter 5).
2. Chronic resistance and endurance trained subjects will show an increased maximal *in vitro* Na^+, K^+-ATPase activity and content (as assessed by [^{3}H]-ouabain binding) compared to control subjects (Chapter 6).
3. Chronic resistance and endurance trained subjects will be characterised by a smaller fatigue-induced depression in maximal *in vitro* Na^+, K^+-ATPase activity associated with improved muscular performance, and a lower [K^+] response during exercise compared to control subjects (Chapter 6).
4. Lung transplant recipients will exhibit diminished maximal *in vitro* Na^+, K^+-ATPase activity and content and this will be associated with an exaggerated rise in plasma [K^+] during exercise (Chapter 7).

Chapter 3 General Methods

3.0 Study Design

This dissertation examined Na^+,K^+-ATPase function in human skeletal muscle. After the development of an assay to measure maximal *in vitro* Na^+,K^+-ATPase activity of human skeletal muscle, the effects of acute and chronic exercise as well as chronic activity were examined. The secondary aspect of this thesis examined a possible relationship between plasma $[K^+]$ during and following exercise in humans and muscle Na^+,K^+-ATPase function.

In the first study, a method was developed to measure Na^+,K^+-ATPase activity in untrained human skeletal muscle (Chapter 4). The second study examined the effects of fatigue on Na^+,K^+-ATPase activity (Chapter 5). The third study utilised a cross sectional design to compare fatigue effects on skeletal muscle Na^+,K^+-ATPase activity in endurance trained and resistance trained athletes in relation to untrained (Chapter 6). The fourth study explored whether muscle Na^+,K^+-ATPase function and K^+ regulation was altered in a group of thoracic transplant recipients, who are characterised by extremely low levels of physical activity (Chapter 7).

3.1 Subjects

Subjects were informed of all test procedures and associated risks before completing a detailed medical questionnaire (Appendix A1) and giving written informed consent (Appendix A2) prior to commencing the study. All experimental protocols were approved by the Victoria University of Technology Human Research Ethics Committee

(Chapters 4, 5, 6, and 7). A total of 24 healthy subjects participated in the studies investigating acute and chronic effects of exercise on Na^+,K^+-ATPase function (Chapters 5, 6). This included 8 untrained (UT) control subjects in which the effects of fatigue were investigated (Chapter 5). The control subjects did not participate in regular physical training but were recreationally active in some cases. These subjects also served as a control group for the third study that involved 8 endurance-trained (ET) and 8 resistance-trained (RT) athletes (Chapter 6). The ET and RT subjects were recruited through the university, gymnasiums, and local competitive clubs and had been involved in resistance or endurance training for at least 2 years. The ET subjects were selected on the basis that their peak oxygen consumption during an incremental exercise test must have at least equalled 60 $ml.min^{-1}.kg^{-1}$. The ET group had typically performed run and/or cycle endurance training for 5-6 hours per week. The RT subjects were selected on the basis of being able to perform a powerlifting-style squat exercise with free weights at least 1½ times their body mass. The RT group had typically trained for approximately 1 hour, 4-5 times per week. Physical characteristics did not differ significantly between the UT, RT and ET subjects (Table 3.1). All subjects were male except for 1 RT female.

Table 3.1 Physical characteristics of untrained, endurance trained and resistance trained subjects

	Untrained	**Endurance trained**	**Resistance trained**
Age (yr)	26.4 ±3.9	26.4 ± 3.1	26.8 ± 7.9
Height (cm)	183.0 ±5.7	177.2 ± 7.1	176.1 ± 4.7
Body mass (kg)	80.4 ±6.8	74.6 ± 9.9	81.6 ± 3.3

Values are n=8, mean ± SD.

The fourth study involved 8 successful thoracic transplant recipients and 8 healthy, age- and gender-matched control subjects (Chapter 7). The thoracic transplant recipients were studied in collaboration with the Department of Respiratory Medicine, and The Heart & Lung Transplant Service, The Alfred Hospital, Melbourne. The Victoria University of Technology Human Research Ethics Committee and The Alfred Group of Hospitals Ethics Committee approved all protocols and procedures (Chapter 7). Physical characteristics did not differ significantly between controls and thoracic transplant recipients (Table 3.2). The mean (±SD) duration post-transplant was 13.5 ± 8.7 months, ranging from 3-24 months after receiving heart /lung (HLTx, n = 2), single-lung (SLTx, n = 2) or double-lung (DLTx, n = 4) transplants. The diagnosis, operation and medication records of the recipients are shown in Table 3.3. No control subjects reported taking any medication.

Table 3.2 Anthropometric data for transplant patients and controls.

	Transplant patients	**Controls**
n (M, F)	8 (3, 5)	8 (3, 5)
Age (yrs)	37.8 ± 10.7	37.3 ± 9.7
Height (cm)	168.3 ± 7.8	169.5 ± 11.7
Body mass (kg)	65.3 ± 11.2	67.1± 12.8

Values mean ± SD

Table 3.3 Clinical characteristics of thoracic transplant patients

Patient	Diagnosis	Opera -tion	Duration Post-operation (mo)	Immunosuppressive Treatment [CsA] (ug/L)	CsA (mg/d)	PNL (mg/d)	AZA (mg/d)
1	PDA+ ES	HLTx	24			25	100
2	IPF	SLTx	15	210	350	15	100
3	PL	SLTx	9	336	300	15	0
4	PPH	HLTx	24	393	450	10	25
5	PPH	DLTx	8	313	400	15	100
6	BO	DLTx	21	198	500	15	100
7	CF+αAT	DLTx	3	222	300	20	75
8	CF	DLTx	4	221	450	15	100

Notes:

Diagnosis:

PDA, patent ductus arteriosis; ES, Eisenmengers Syndrome; PPH, primary pulmonary hypertension; PL, pulmonary lymphangiolieomatosis; IPF, idiopathic pulmonary fibrosis; CF, Cystic Fibrosis; αAT, α_1-anti-trypsin deficiency; BO, bronchiolitis obliterans.

Medication:

1. Immunosuppressive medication was used in virtually all post transplant recipients to prevent graft rejection: CsA, Cyclosporine A; [CsA] CsA concentration in blood, PNL, Prednisolone; AZA, Azathioprine. Corticosteroids.

2. Other major medications included: Antihypertensive drugs: calcium channel blockers, Verapamil and Nifedipine; angiotensin-converting enzyme inhibitors: enalapril, captopril, Omeprazole, Ranitidine; Calcium, Potassium and Magnesium supplements.

3.2 Test procedures

All subjects in the second and third studies (Chapters 5 and 6) reported to the laboratory for four test sessions, with exercise tests separated by a minimum of 3, and a maximum of 14 days. The four tests involved anthropometric measurements, a muscle function test on a Biodex isokinetic dynamometer, a muscle fatigue test with muscle biopsies and arterialised-venous blood samples on a Cybex isokinetic dynamometer. The subjects were tested in the Human Performance Laboratory at the Footscray campus, except for the Biodex muscle function test, which was conducted in the Biomechanics laboratory at the City Campus. The fourth test involved an incremental $\dot{V}O_2$ peak test on a cycle ergometer with arterialised-venous blood samples. The transplant recipients, and thus age-matched controls, described in Chapter 7, did not perform the muscle function or fatigue tests for ethical reasons.

3.2.1 Anthropometric Measurements

All subjects were measured for height using a stadiometer (Novel Inc, Addison, Illinois, U.S.A) and body mass recorded with a sensitivity of 0.005 kg on an electronic scale (Sauter E1200, West Germany). Subcutaneous skinfold thickness was measured at 8 sites: tricep, bicep, subscapular, mid-axilla, suprailiac, abdominal, anterior thigh and medial calf, using Harpenden calipers (British Indicators, W. Sussex, United Kingdom). Sum of 8 skinfold thickness was determined and from body density using the following regression equations (Jackson & Pollock 1978; Jackson et al. 1980; Siri 1956).

The regression equation for men and women are shown in Equation 3.1 & 3.2 respectively.

Equation 3.1

$BD = 1.112000 - 0.0004399\ (X_1) + 5.5\ 10^{-7}(X_1)^2 - 0.00028826(X_2)$

where BD = body density, X_1 = sum triceps, biceps, mid-axilla, subscapular, abdominal thigh and calf skinfolds, X_2 = age (years)

Equation 3.2

$BD = 1.096095 - 0.0006952\ (X_1) + 1.1*10^{-6}(X_1)^2 - 0.0000714(X_2)$

where BD = body density, X_1 = sum triceps, abdominal supra-iliac, and thigh skinfolds (mm), X_2 = age (years)

The % body fat was calculated using the Siri equation

Equation 3.3

$\%\ body\ fat = [(4.95/BD) - 4.50\ ] *100$

Estimation of thigh volume for the muscle fatigue tests was determined anthropometrically using limb lengths and girths (Jones & Pearson 1969). With subjects standing erect and feet slightly apart, girths were measured with a flexible tape measure while segmental lengths were determined with an Harpenden anthropometer (Holtain, Dyfed, United Kingdom) to an accuracy of 1mm. Thigh muscle and bone cross sectional area was calculated from circumference and skinfold measures with these and the results for all subject's skinfold and limb volume measurements shown in Appendix B1: Tables B1.1-B1.5.

3.2.2 Lung Function Testing

All subjects performed spirometry to determine their vital capacity (VC) and forced-expired volume in 1 second (FEV_1). Healthy subjects were tested on a spirometer

(Minato, Osaka, Japan) whilst transplant recipients were tested on a Jaeger Masterlab 3.30 spirometer (Wuerzburg, W. Germany), but both spirometers were not cross-validated. The results for all groups and comparison to predicted values (Crapo et al. 1981) are shown in Appendix B1.

3.2.3 Maximal Aerobic Power

Each subject refrained from vigorous exercise, alcohol and caffeine consumption for 24 hours prior to the test. All subjects had resting heart rate and blood pressure recorded. Subjects performed an incremental exercise test on an electrically braked cycle ergometer (Lode N.V. Groningen, Netherlands) to determine peak oxygen consumption ($\dot{V}O_2$ peak). Subjects maintained 60-80 rpm with workrate increased by 25 W each min until volitional exhaustion, defined as an inability to maintain pedal cadence above 50 rpm. Subjects breathed through a Hans-Rudolph two-way non-rebreathing valve, with expired air passing through low-resistance plastic tubing into a 4 L mixing chamber. Expired volume was measured using a flow transducer (KL Engineering, Sunnyvale, California, USA), and mixed expired oxygen and carbon dioxide contents were analysed by rapidly responding gas analysers (Applied Electrochemistry S-3A O_2 and CD-3A CO_2, Ametek, Pittsburgh, USA). Ventilatory gas data were calculated and averaged over 15s (TurboFit, California USA). The gas analysers were calibrated immediately prior to each test, and rechecked after the test, using commercially prepared gas mixtures. The ventilometer was calibrated prior to each test using a standard 3 L syringe. A 6-lead ECG was used at rest, during and following exercise to determine heart rate and monitor heart rhythm (Mortara, X- Scribe, Milwaukee, USA). Arterialised venous blood samples were collected from a dorsal hand vein at rest, each

min during graded exercise and at 1, 2, 5, 10, 20, and 30 min in recovery. Arterialisation was obtained by heating the hand as described in section 3.4.

3.2.4 Muscle Function Assessment

To characterise muscle function of the different subject groups, maximal muscle strength and fatigability of the right knee extensor muscle group was determined in two separate tests on an isokinetic dynamometer (Biodex Medical Systems, New York, USA). Prior to testing, subjects completed a 3 min warm up at 50W on an air- braked cycle ergometer. Subjects were then strapped to the Biodex chair using belts across the hips and chest, to restrict movement of upper body muscles, and across the thigh to stabilise the leg.

3.2.4.1 Muscle torque-velocity Relationship

The first test measured leg peak muscle torque at a series of limb velocities, from which a torque-velocity relationship was constructed. Subjects performed 2 practice maximal repetitions, followed by 1 min rest. Then 3 maximal repetitions were performed in order at each of 60, 120, 180, 240, 300 and 360 °/s, each separated by 2 min rest. After the 360°/s contraction, subjects rested for 3 min before being tested for isometric peak torque (0°/s). Peak torque was expressed in Nm, relative to body mass, and relative to an approximate measure of the lever arm, height (Schantz et al. 1983).

3.2.4.2 Muscle fatigue test

After 30 min recovery subjects completed a standard test designed to induce local fatigue of the knee extensor muscles, involving 50 repetitions of maximal knee extension (180°/s, 0.5Hz), modified from Thorstensson et al. (1976). The peak torque during the test was defined as the mean of the 5 strongest in the first 10 contractions. The final peak torque during the test was defined as the mean of the 5 weakest in the final 10 contractions. The fatigue index, expressed as a percentage, was calculated as

the decline in power from the initial five strongest to the final five weakest contractions (Equation 3.4):

Equation 3.4: Fatigue Index = $100 \times \left(\frac{\text{Peak Torque-Final Torque}}{\text{Peak Torque}} \right)$

3.2.5 Muscle Fatigue Test with invasive measures

Each subject refrained from vigorous exercise, alcohol and caffeine consumption for 24 hours prior to an invasive muscle fatigue test performed on an isokinetic dynamometer (CybexII Lumex, Ronkoukowany, USA). Separate dynamometers were used for practical reasons. The 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 could 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.

Muscle biopsies were taken at rest and immediately at the end of exercise while blood samples were taken at rest, during and following exercise (Figure 3.2). Subjects were strapped to the Cybex dynamometer chair using velcro belts to restrict upper body movement (Figure 3.1). As described in the Biodex fatigue test subjects were required to perform 50 maximal knee extensions (MFT 180°/s, 0.5Hz, Chapters 5 and 6). Peak torque and fatigue index was calculated as described for the Biodex fatigue test. Data was expressed in Nm and work done calculated from the area under the torque displacement curve.

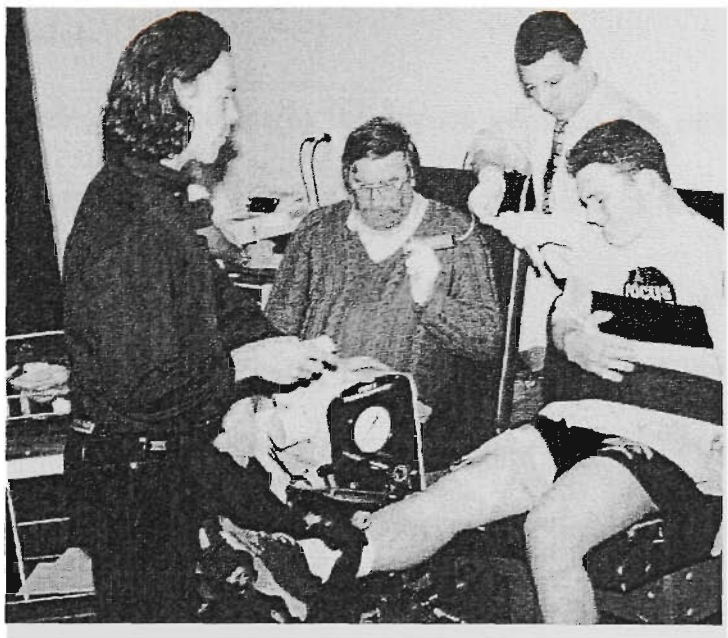

Figure 3.1 Subject performing maximal knee extensions on a Cybex dynamometer with biopsy ready to be taken from vastus lateralis.

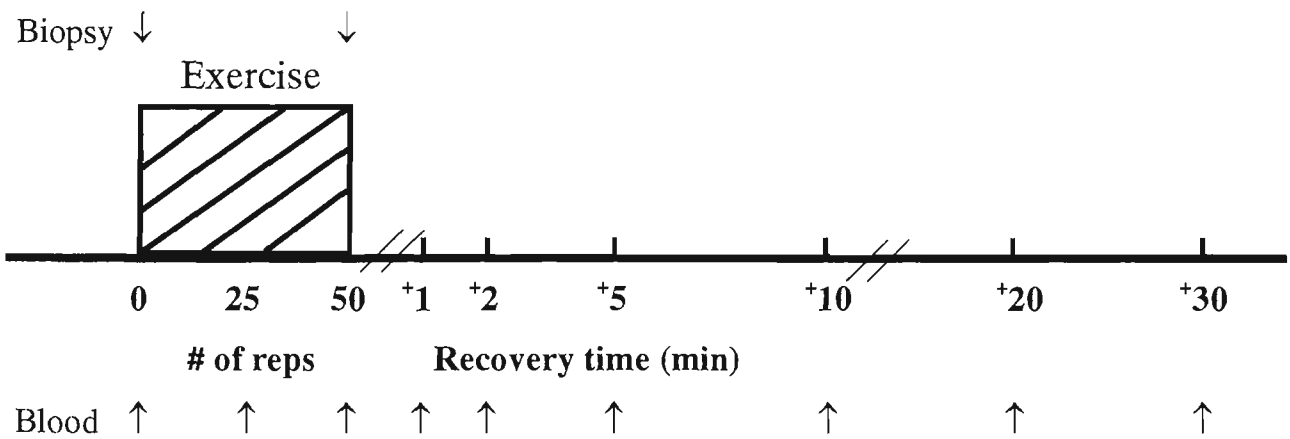

Figure 3.2 Blood and muscle sampling during fatigue test; Exercise: 50 maximal knee extensions on Cybex isokinetic dynamometer (180°/s, 0.5Hz). ↑ denotes blood sampling and ECG recording; ↓ denotes muscle biopsy.

3.3 Muscle Biopsy Sampling

3.3.1 Muscle Biopsies

Three muscle biopsies were taken in the supine position for each subject in Studies 2 and 3, comprising two biopsies at rest and one at the point of fatigue (Chapters 5 and 6). Two biopsies were taken at rest for subjects in Study 4 (Chapter 7). After injection of a local anaesthetic into the skin and fascia (1% Lignocaine), a small incision was made in the mid-portion of the vastus lateralis muscle of the right leg. Muscle samples (40 -120 mg) were taken, at a constant depth, using the percutaneous needle biopsy technique with suction (Bergström, 1962; Evans et al. 1982). Resting biopsies were analysed for muscle fibre type, Na^+,K^+-ATPase activity and content, as well as for substrate and metabolite contents. The fatigue biopsy sample was analysed for muscle Na^+,K^+-ATPase activity, as well as for substrate and metabolite content. Immediately after excision, muscles were separated into portions, with one portion rapidly frozen and stored in liquid nitrogen for subsequent metabolite and substrate determination. The remaining portion was blotted on filter paper, weighed, homogenised, and frozen in liquid nitrogen for subsequent determination of Na^+,K^+-ATPase activity.

3.3.2 Muscle Morphology.

Approximately 10-15 mg was separated from the resting biopsy sample and was mounted using an embedding medium (Jung Embedding medium, Nussloch, Germany), quick-frozen in isopentane precooled in liquid nitrogen, and stored in liquid nitrogen until analysis. Muscle fibre types were analysed using the myofibrillar ATPase method (Brooke & Kaiser 1970). Fibre typing was performed on 10μm thick sections of muscle cut on a cryostat at -20° C (Microm GMBH D-6900 500, Heidelberg, Germany). Fibres were classified into type I (slow-twitch) and type IIA (fast oxidative) and IIB

(fast-glycolytic) according to their myofibrillar ATPase staining pattern after pre-incubation at pH 10.3, 4.6 and 4.3.

3.3.3 Muscle Homogenisation

Muscle samples (30-40 mg) were immediately blotted on filter paper, weighed, then homogenised (5% w/v) at 0°C for 2 x 20s, 20000 rpm (Omni 1000, Omni International, Gainesville, Virginia, U.S.A.) in an homogenate buffer containing 250 mM sucrose, 2 mM EDTA, and 10 mM Tris (pH 7.40). Muscle homogenates were immediately frozen and stored in liquid N_2 until 3-*O*-MFPase activity determination.

3.3.4 3-*O*-MFPase Assay

Skeletal muscle Na^+,K^+-ATPase activity was determined using the 3-*O*-MFPase assay, modified from two previously established techniques for rat (Nørgaard et al. 1984) and human skeletal muscle (Benders et al. 1992). Development of this technique is described in Chapter 4. Before analysis, homogenates were freeze-thawed four times, then diluted 1/5 in cold homogenate buffer. The assay medium in which 3-*O*-MFPase activity was measured contained 5 mM $MgCl_2$, 1.25 mM EDTA, 100 mM Tris, and an 80 nM 3-*O*-MF standard (pH 7.40). The freeze-thawed, diluted homogenate (30µl) was incubated in 2.5ml of assay medium at 37°C for 5 min before addition of 40µl of 10 mM 3-*O*-MFP to initiate the reaction. After 60 seconds, 10µl of 2.58 M KCl (final concentration 10 mM) was added through an injection port to stimulate K^+-dependent phosphatase activity, with the reaction measured for another 60 seconds. The K^+-stimulated 3-*O*-MFPase assays were performed at 37°C, using continuous stirring, on a spectrofluorimeter (Aminco Bowman AB2 SLM, Urbana, Illinois, U.S.A). Excitation wavelength was set at 475nm and emission wavelength 515nm, with 4nm slit widths. To

calculate the K^+-stimulated 3-*O*-MFPase activity, the unspecific ATPase activity and spontaneous hydrolysis present before the addition of 10 mM KCl was subtracted from the activity obtained after KCl addition. Protein content of the homogenate was determined spectrophotometrically using bovine serum albumin as a standard (Lowry et al. 1951).

3.3.5 Muscle Na^+,K^+-ATPase content

Approximately 20mg of the frozen resting muscle for all subjects in studies 2-4 was used to determine the Na^+,K^+-ATPase content using the [^{3}H]-ouabain binding method (Kjeldsen, 1986; Nørgaard et al. 1984a). All analyses were performed by the author and were performed in collaboration with the Medical Department B, Rigshospitalet, Copenhagen Denmark. Each sample was packed in dry ice for shipment to Denmark. Samples were cut into small pieces of 2-4 mg wet weight. In all experiments freshly made vanadate solution was used.

Samples were washed at 0°C for 20 min, with a change of medium after 10 min (2 x10 min) in a buffer consisting of 10 mM tris (hydroxymethyl)aminomethane chloride, 250mM sucrose, 3mM $MgSO_4$, and 1 mM vanadate, pH 7.2-7.4. This procedure was used to thaw the samples, preincubate them with vanadate and to maintain low Na^+ and K^+ concentrations that would not interfere with vanadate-facilitated [^{3}H]-ouabain binding. Subsequent incubations for vanadate-facilitated [^{3}H]-ouabain binding took place in a buffer containing 2 μCi/ml [^{3}H]-ouabain, and ouabain added to a final concentration of 1 μM at 37°C for 2 hours with a change of medium after 1 hour. After incubations, a washout at 0°C in unlabeled buffer for 2 hours with a change of medium every 30 min (4 x 30min) was performed to reduce the [^{3}H]-ouabain in the extracellular space and enhance the precision of the method. After washout, samples were blotted on

dry filter paper, weighed, and soaked overnight in minivials containing 0.5 ml of 5% trichloroacetic acid (TCA). The next day 2.5 ml of scintillator (Opti-fluor®) was added before liquid scintillation counting of the [^{3}H]-ouabain activity was performed. The amount of ^{3}H ouabain taken up and retained by the samples was calculated on the basis of the sample wet weight and the specific activity of the incubation medium and samples. Results are given as picomoles per gram wet weight.

3.3.6 Muscle metabolites and pH.

Muscle was freeze dried, dissected free of any connective tissue, weighed, powdered and extracted (Harris et al. 1974). Approximately 2mg of freeze dried muscle was extracted in PCA (0.5 M) and neutralized in $KHCO_3$ (2.1 M). Muscle extracts were analysed for ATP, CP, glycogen and creatine contents using fluorimetric techniques (Model 112 Turner Fluorometer California, USA) and for lactate content using spectrophotometric techniques (Shimadzu UV-120-02. Japan) according to the methods of Lowry and Passonneau (1972). Reverse-phase high performance liquid chromatography (HPLC) was used to quantify ADP, AMP, and IMP contents (Wynants & Van Belle 1985). Muscle metabolites excepting glycogen and lactate were corrected for total creatine content. Muscle homogenate pH was determined in 2-4 mg freeze-dried tissue (1mg/100µl) with a pH microelectrode (MI-410/ Microelectrode Inc., USA) at 37 °C, in an homogenate buffer which contained sodium iodacetate (5 mM), KCl (145 mM) and NaCl (10 mM, Spriet et al. 1989).

3.4 Blood Analyses

3.4.1 Blood sampling

A catheter (20G, Jelco) was inserted into a superficial dorsal hand vein prior to the muscle fatigue test as well as the incremental aerobic power test. All blood samples were arterialised by heating the left hand in a hot (45°C) water bath for 10 min before samples were taken (McLoughlin et al. 1992). Two syringes of blood were drawn at each sampling time, the first for plasma gas and electrolyte concentrations (1.0 ml, Rapidlyte, Ciba Corning Diagnostics Corporation, Medfield, USA); the second for haematocrit and lactate measurements (5 ml, Terumo Medical Corporation, Elkton, USA). The catheter was kept patent by periodic infusions of heparinised isotonic saline (1 ml).

3.4.2 Blood processing and analyses

The blood was mixed well, air bubbles removed from the syringe, capped tightly and placed on ice for subsequent analysis of plasma acid-base status and gas tensions (pH, pCO_2, pO_2), and electrolyte concentrations (K^+, Na^+, Cl^-) in duplicate on an automated analyser (865 Ciba Corning, Medfield, USA).

Haemoglobin concentration ([Hb]) was determined in duplicate spectrophotometrically (Radiometer OSM2, Copenhagen, Denmark). From the second syringe 1ml of blood was portioned into an eppendorf tube and analysed in triplicate for haematocrit (Hct). Samples were spun for 8 minutes in a microcentrifuge (Hettich Zentrifugen D-7200, Tuttlingen, Germany, CV for Hct was 0.6%). All analytical instruments were calibrated before and during the analyses with precision standards.

A 1 ml aliquot of whole blood was centrifuged at 4000 rpm for 4 min, and the plasma separated. A 200µl aliquot of both plasma and whole blood were separately

deproteinised in 600 µl of cold 3M perchloric acid, vortexed, centrifuged at 4000 rpm for 4 min, the supernatant drawn off, then stored at -80°C. Blood and plasma lactate were later analysed in triplicate, using an enzymatic spectrophotometric technique (Lowry & Passonneau 1972).

3.4.3 Calculations

pH was converted to $[H^+]$ using equation 3.5

Equation 3.5 $[H^+] = 1 \times 10^{(-pH)}$,

The rise in plasma $[K^+]$ above rest ($\Delta[K^+]$) and the ratio of the rise in $[K^+]$ divided by the work done ($\Delta[K^+]$/ work, McKenna et al. 1993) were also calculated (Equation 3.6 and 3.7).

Equation 3.6 $\Delta\ [K^+]\ (mM) = [K^+] - [K^+]rest$

Equation 3.7 $\Delta[K^+]/Work\ (nM.J^{-1}) = \Delta\ [K^+] / Work\ Done$

The decline in K^+ from peak exercise at one, two and five min recovery was also determined. The decline in plasma volume ($\Delta PV\%$) during exercise and recovery was calculated from changes in Hct and [Hb] (Equation 3.8, Edwards & Harrison 1984).

Equation 3.8 $$\%\Delta PV = 100^* \left(\frac{[Hb]_1 \times (1-Hct_2)}{[Hb]_2 \times (1-Hct_1)} \right)$$

3.5 Statistical Analyses

Data are presented as mean ± standard error of the mean (SEM) except where stated otherwise. Statistical analyses were performed by paired *t* tests (eg Rest vs Fatigue, Chapter 5), or one-way analysis of variance for two variable comparisons (Chapter 6) whilst for non-paired data, an independent t-test was employed (eg transplant patient vs age-matched control, Chapter 7). A two-way analysis of variance (ANOVA) with

repeated measures on one variable was used where there were more than 2 variables compared. When a significant F-ratio was obtained in ANOVA procedures, data were further analysed with a Newman-Keuls post-hoc test. Relationships among variables were established by least squares linear regression analyses. Significance was accepted at an α-level of 0.05.

Chapter 4 Development of a method for measurement of Na^+,K^+-ATPase activity in human skeletal muscle

4.0 Introduction

During skeletal muscle membrane excitation, each action potential is associated with sodium (Na^+) influx and potassium (K^+) efflux (Hodgkin & Horowitz 1959). The gain in intracellular [Na^+] extracellular [K^+] are minimised by the Na^+, K^+-ATPase enzyme during repeated excitation of muscle (Clausen 1990). The rapid activation of the Na^+, K^+-ATPase enzyme in contracting skeletal muscle therefore helps preserve muscle membrane potential, excitability, and reduces muscular fatigue (Everts & Clausen 1994).

The capacity to regulate excitation-induced Na^+/K^+ exchange depends on the total content, as well as the maximal activity of the Na^+, K^+-ATPase enzyme. Although most authors have focussed on factors regulating the total content of Na^+, K^+-ATPase in muscle (Clausen 1996a; McKenna et al. 1993), it is apparent that under certain conditions, the maximal enzyme activity may be altered independent of the total content (Druml et al. 1988; Pickar et al. 1994). Thus, to quantitate muscle Na^+/K^+ exchange capacity it is necessary to measure both maximal *in vitro* Na^+,K^+-ATPase activity and the total content of Na^+,K^+-ATPase.

The total content of Na^+,K^+-ATPase is best quantified by [^{3}H]-ouabain binding measurements, as reviewed elsewhere (Nørgaard 1986). Perhaps the best quantitative method of maximal Na^+,K^+-ATPase activity is through measurement of radiolabelled

ion fluxes and this technique has been applied during electrical stimulation of isolated intact muscle preparations (Clausen et al. 1987). However, these techniques cannot be used to measure maximal Na^+,K^+-ATPase activity in human skeletal muscle samples obtained by needle biopsy. Biochemical methods used to quantify the maximal *in-vitro* Na^+,K^+-ATPase activity in skeletal muscle samples include measurements of inorganic phosphate production in purified preparations (Bonting et al. 1961), or the activity of related K^+-dependent phosphatases, using substrates such as *p*- nitrophenyl phosphate (pNpp, Hundal et al. 1994; Judah et al. 1962), or 3-*O*-methylfluorescein phosphate (3-*O*-MFP, Kjeldsen et al. 1988).

The use of purification procedures inevitably results in extremely low recovery of enzyme activity and therefore should not be used for quantifying total Na^+,K^+-ATPase activity (Hansen & Clausen 1996; Nørgaard et al. 1984b). In contrast, complete membrane and enzyme recovery can be attained using the K^+-stimulated pNppase and 3-*O*-MFPase assays in rat muscle homogenates (Hansen & Clausen 1996). The K^+-stimulated 3-*O*-MFPase assay has a 2-3 times higher sensitivity, therefore requiring 50-100 times less tissue, than the K^+-stimulated pNppase (Clausen et al. 1987). Thus, the K^+-stimulated 3-*O*-MFPase assay is the preferred method for studies investigating Na^+,K^+-ATPase activity in small human muscle samples obtained by needle biopsy.

To our knowledge there are only two reports of K^+-stimulated 3-*O*-MFPase activity in human skeletal muscle (Benders et al. 1992, 1993), based on an earlier method for rat skeletal muscle (Nørgaard et al. 1984b). However, during preliminary investigations in our laboratory, we were unable to reliably determine K^+-stimulated 3-*O*-MFPase activity in human skeletal muscle using this technique. The present paper describes several substantial modifications to earlier K^+-stimulated 3-*O*-MFPase activity assays

(Benders et al. 1993; Kjeldsen et al. 1988) to optimise enzymatic conditions for measurement of Na^+,K^+-ATPase activity in human skeletal muscle.

Five experiments were conducted using the revised K^+-stimulated 3-*O*-MFPase assay in crude human muscle homogenates. The first two experiments determined the optimal substrate and the K^+-stimulating concentrations. The third experiment confirmed that maximal enzyme exposure, and therefore maximal activity, was obtained using a freeze-thaw treatment of the homogenate, whilst the fourth experiment assessed the inter- and intra- assay variability in human skeletal muscle. The final experiment investigated the specificity of the K^+-stimulated 3-*O*-MFPase assay using the Na^+,K^+-ATPase inhibitor, ouabain. On the basis of these experiments we report an improved and reliable K^+-stimulated 3-*O*-MFPase method suitable for the determination of *in vitro* Na^+,K^+-ATPase activity in human skeletal muscle.

4.1 Methods

4.1.1 Chemicals and Stock Solutions

All chemicals were of analytical grade with 3-*O*-methylfluorescein, 3-*O*-methylfluorescein phosphate, and ouabain obtained from Sigma Chemicals, St. Louis, Missouri, USA. A 4 mM 3-*O*-methylfluorescein standard solution was prepared in methanol. A 10 mM 3-*O*-methylfluorescein phosphate solution was prepared in assay medium with pH initially adjusted to 12.0 to allow 3-*O*-MFP to dissolve before readjustment of pH back to 7.40.

4.1.2 Needle biopsy and preparation of muscle homogenates:

Ethical approval was obtained from the Human Research Ethics Committee at Victoria University of Technology. After giving written informed consent, seven

healthy, untrained male subjects volunteered for the study (mean ± SD, age 29.7 ± 7.6 yrs, height 177.1 ± 9.2 cm, body mass 79.4 ± 14.2 kg). Whilst resting supine on a couch, subjects had biopsies taken from the vastus lateralis muscle under local anaesthesia (1% lignocaine injection), using the percutaneous needle biopsy technique (Bergström 1962). Muscle samples (30-40 mg) were immediately blotted on filter paper, weighed, then homogenised (5% w/v) at 0°C for 2 x 20s, 20000 rpm (Omni 1000, Omni International, Gainesville, Virginia, U.S.A.) in an homogenate buffer containing 250 mM sucrose, 2 mM EDTA, and 10 mM Tris (pH 7.40). Muscle homogenates were immediately frozen and stored in liquid N_2 until analysis.

4.1.2 Assay

Before analysis, homogenates were freeze-thawed four times, then diluted 1/5 in cold homogenate buffer. The assay medium in which 3-*O*-MFPase activity was measured contained 5 mM $MgCl_2$, 1.25 mM EDTA, 100 mM Tris, and an 80 nM 3-*O*-MF standard (pH 7.40). The freeze-thawed, diluted homogenate (30µl) was incubated in 2.5ml of assay medium at 37°C for 5 minutes before addition of 40µl of 10 mM 3-*O*-MFP to initiate the reaction (Fig 4.1). After 60 seconds, 10µl of 2.58 M KCl (final concentration 10 mM) was added through an injection port to stimulate K^+-dependent phosphatase activity, with the reaction measured for another 60 seconds. The K^+-stimulated 3-*O*-MFPase assays were performed at 37°C, using continuous stirring, on a spectrofluorimeter (Aminco Bowman AB2 SLM, Urbana, Illinois, U.S.A). Excitation wavelength was set at 475nm and emission wavelength 515nm, with 4nm slit widths.

4.1.3 Calculation of Na^+,K^+-ATPase activity

To calculate the K^+-stimulated 3-*O*-MFPase activity, the unspecific ATPase activity and spontaneous hydrolysis present before the addition of 10 mM KCl was subtracted from the activity obtained after KCl addition. Protein content of the homogenate was determined spectrophotometrically (Lowry et al. 1951).

4.1.4 Optimising assay conditions

Four experiments were conducted to optimise conditions for the 3-*O*-MFPase assay for human skeletal muscle. Firstly the optimal substrate concentration was tested by measuring maximal K^+-stimulated 3-*O*-MFPase activity at 0, 20, 40, 100, 160, 200, and 400μM 3-*O*-MFP. Secondly the optimal K^+-stimulating concentration was determined by measurement of 3-*O*-MFPase activity after the addition of 0, 2.5, 5.0, 10, 15, 20, and 25 mM KCl. The extent to which latent activity was exposed in this preparation was tested with four freeze-thaw cycles, compared to a non freeze-thaw treatment of the homogenate. Inter- and intra-assay variability were also reported.

4.1.5 Assay Specificity

The final experiment was to confirm the assay specificity for the Na^+,K^+-ATPase enzyme. Ouabain was added to the homogenate at a final concentration of 1 mM and incubated at 37°C for either 10 or 30 minutes.

4.1.6 Statistics

Data are presented as mean ±sem. Statistical analyses were performed by one-way analysis of variance, with post-hoc analyses using the Student Newman-Keuls test.

Paired t-tests were performed for the freeze-thaw experiment. Significance was accepted at $P< 0.05$.

4.2 Results

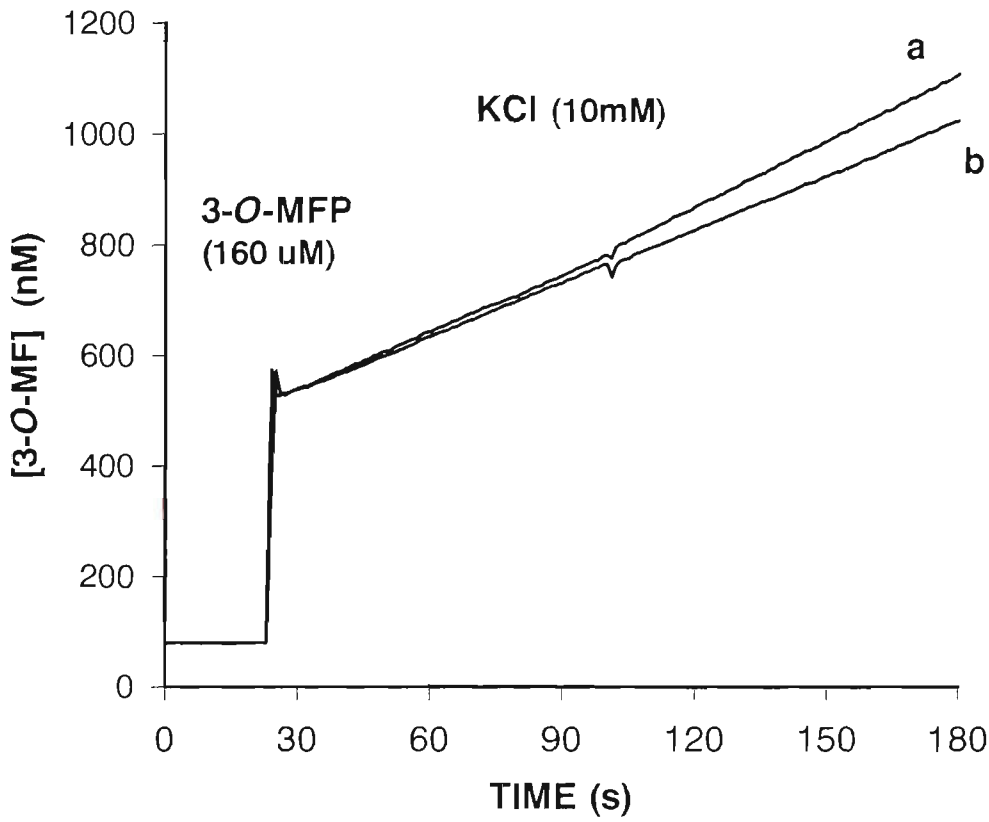

Figure 4.1 (**A**) A typical K^+-stimulated 3-*O*-MFPase assay in a crude human skeletal muscle homogenate. The reaction was initiated by the addition of 160 μM 3-*O*-MFP at 20 s, with K^+-stimulated 3-*O*-MFPase activity induced by addition of 10 mM KCl at 100 s. (**B**) Superimposed is a second assay with homogenate previously incubated with 1 M ouabain for 10 min. Note that K^+-stimulated activity was abolished by ouabain. Activity is calculated by the difference in the two slopes in A.

4.2.1 Substrate velocity curve

The homogenate 3-O-MFPase activity progressively increased when 3-O-MFP was increased from 19.5 μM to 160 μM (P <0.05), but was not significantly different at higher substrate concentrations (Fig 4.2). Thus, an optimal 3-O-MFP concentration of 160 μM was chosen.

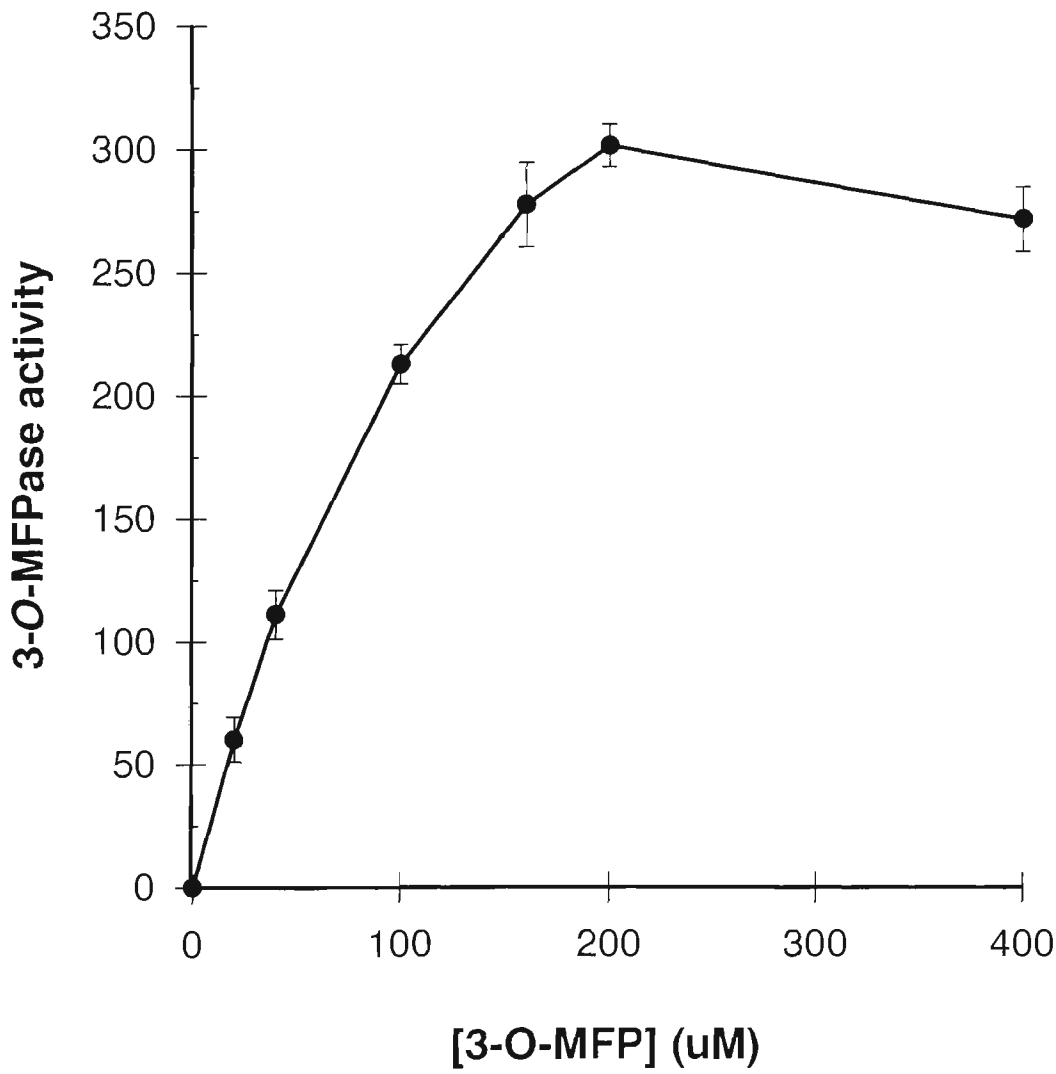

Figure 4.2 Substrate-velocity curve for 10 mM K^+-stimulated 3-*O*-MFPase activity (nmol min^{-1} g^{-1} wet wt) in crude human skeletal muscle homogenate (mean±SEM, n=3). The optimal [3-*O*-MFP] was 160 μM.

4.2.2 Optimal K^+ concentration

The K^+-stimulated 3-*O*-MFPase activity (using 19.5μM [3-*O*-MFP]) increased as the KCl concentration increased from 0 to 10 mM (P <0.05), did not differ from 10 mM at 15 and 20 mM KCl, and was lower than 10 mM at 25 mM KCl (P <0.05, Fig. 4.3). Thus 10 mM KCl was chosen as the optimal K^+-stimulating concentration. Substrate concentration used throughout these experiments was 160 μM 3-*O*-MFP.

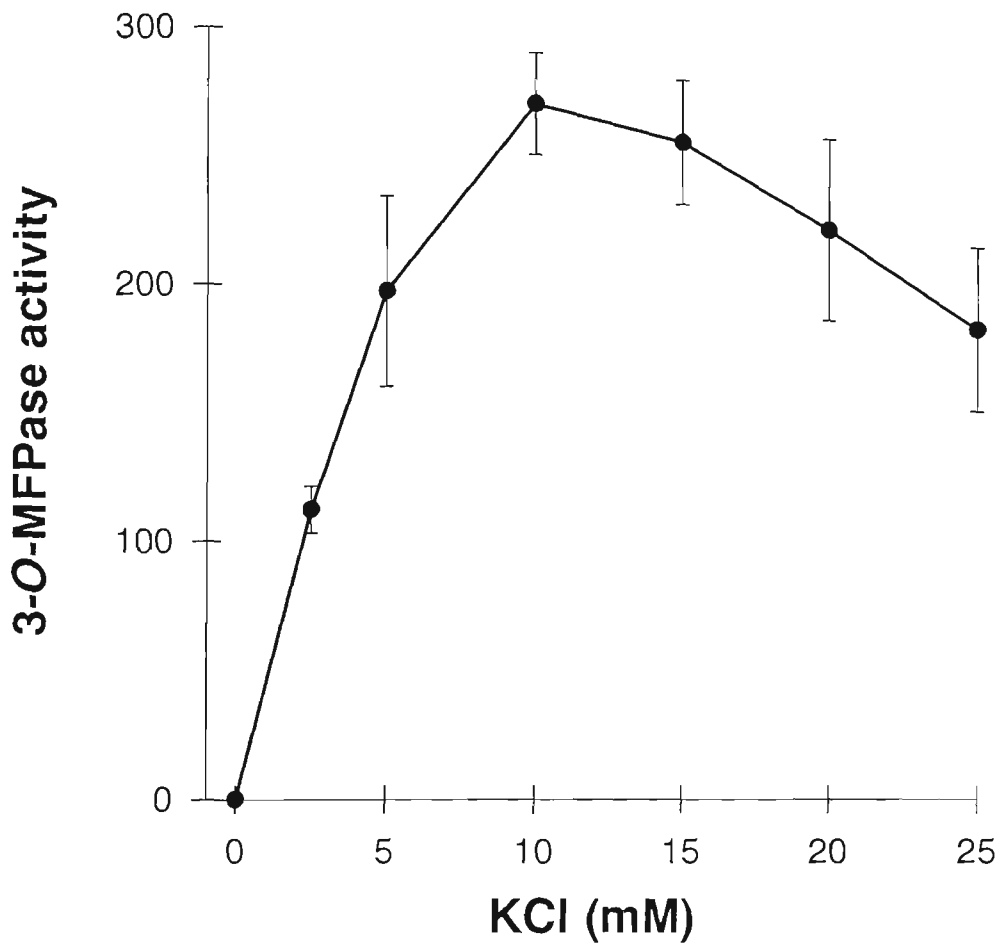

Figure 4.3 Effect of different KCl concentrations on K^+-stimulated 3-*O*-MFPase activity (nmol min^{-1} g^{-1} wet wt) in crude human skeletal muscle homogenate (mean±SEM, n=3). Using 160μmol [3-*O*-MFP], the optimal KCl concentration was 10 mM.

4.2.3 Exposing latent activity

The four freeze-thaw cycles increased K^+-stimulated 3-*O*-MFPase activity by 24%, compared to a non freeze-thawed homogenate (P <0.05).

4.2.4 Assay precision

The inter-assay variation (same homogenate on different days) for the 3-*O*-MFPase assay conducted on human skeletal muscle homogenate was 5.3% (n=5, mean 242±13 nmol min^{-1} g^{-1}). The intra-assay (repeated analysis of same homogenate) variation was 8.1% (n=6, mean 246±20 nmol min^{-1} g^{-1}).

4.2.5 Assay specificity

The ouabain inhibition of K^+-stimulated 3-*O*-MFPase activity is shown in Figure 4.1. Ten and thirty minute incubation with 1 mM ouabain inhibited K^+-stimulated 3-*O*-MFPase activity by 85± 3% and 97± 2% respectively (n=3).

4.2.6 Human skeletal muscle K^+-stimulated 3-O-MFPase activity

Using the optimised conditions described above, the maximal K^+-stimulated 3-*O*-MFPase activity in seven human skeletal muscle samples was 292 ± 10 nmol min^{-1} g^{-1}, or 1745 ± 84 pmol min^{-1} mg^{-1} protein.

4.3 Discussion

This study reports an improved K^+-stimulated 3-*O*-MFPase assay that allows valid and reliable measurement of Na^+,K^+-ATPase activity in human skeletal muscle. The K^+-stimulated 3-*O*-MFPase activity in muscle obtained by needle biopsy from young

untrained males was 292 nmol min^{-1} g^{-1}, which was five-fold higher than the only other published results for human skeletal muscle (Benders et al. 1992, 1993). The current assay optimised enzymatic conditions for human skeletal muscle and was modified from previous work using rat (Kjeldsen et al. 1988) and human (Benders et al. 1992) skeletal muscle.

The most important modification resulted from our finding that the optimal substrate concentration for the K^+-stimulated 3-*O*-MFPase assay was 160 μM. This was eight times higher than the previously used concentration of 19.5 μM, based on a purified enzyme preparation from rat skeletal muscle (Kjeldsen et al. 1988). Previous work had assumed 19.5 μM 3-*O*-MFP to be optimal for human skeletal muscle (Benders et al. 1992). The 5-fold higher activity reported for human muscle in the present study can largely be attributed to this modification. Further increases in [3-*O*-MFP] above 160 μM were avoided as this promoted an increased rate of spontaneous 3-*O*-MFP hydrolysis, that may mask the 3-*O*-MFPase activity specific to the Na^+,K^+-ATPase enzyme.

The major reason for the development of this assay was to overcome large inter and intra assay variability using the previous technique which compared K^+-stimulated 3-*O*-MFPase activity in one run against ouabain inhibited activity in another run (Benders et al. 1992). In the present assay, the K^+-stimulated 3-*O*-MFPase activity was determined in a single run, and this substantially reduced variability. We report low variability for the K^+-stimulated 3-*O*-MFPase assay, thus confirming the suitability of this assay for reliable measurement of Na^+,K^+-ATPase activity in human skeletal muscle. The inter-assay variation (between different homogenates) for the 3-*O*-MFPase assay conducted on human skeletal muscle homogenate was 5.3% (n=5,

mean 242 nmol min^{-1} g^{-1}). The intra-assay (repeated analysis of same homogenate) variation was 8.1% (n=6, mean 246 nmol min^{-1} g^{-1}).

Two important aspects of the 3-*O*-MFPase assay were confirmed for human skeletal muscle in this study. Firstly, it was clearly established that the modified 3-*O*-MFPase assay was specifically measuring Na^+,K^+-ATPase activity, with complete inhibition of activity after 30 min of incubation with ouabain. The greater inhibition after 30 compared to 10 min incubation, is consistent with other findings of slow ouabain inhibition (Huang & Askari 1975), and would also partially account for the higher activity reported in this study, compared to the 10 minute incubation used in the previous assay (Benders et al. 1992). Secondly, the optimal K^+ activation for human skeletal muscle was achieved with 10 mM KCl, in agreement with other substrate assays for Na^+,K^+-ATPase (Izumi et al. 1966; Pitts & Askari 1971). Interestingly, the optimal K^+-stimulating concentration is equivalent to the peak muscle interstitial [K^+] reported during maximal muscular contractions in humans (Vyskocil et al. 1983).

Unmasking latent activity concealed in vesicles can be achieved if the vesicles are broken into membrane fragments to allow substrate and ligand access to the catalytic site of the enzyme. Breaking these vesicles and unmasking latent 3-*O*-MFPase activity could be performed with detergent treatment (Jorgensen et al. 1971) or by freeze-fracture (Benders et al. 1992). Using inorganic phosphate production as a measure of maximal Na^+,K^+-ATPase activity, Benders et al. (1992) showed that detergent treatment of cultured human muscle cells did not increase Na^+,K^+-ATPase activity, but did show an increase in activity when the samples were freeze thawed before analysis. However they did not report what effect these different treatments had on muscle biopsy homogenate Na^+,K^+-ATPase activity using the 3-*O*-MFPase assay. The present study showed that four freeze thaw cycles increased activity,

consistent with increases seen in Na^+,K^+-ATPase activity shown by Benders et al. (1992). The use of SDS detergent also increased activity but the results were inconsistent (data not shown). Inconsistency could be due to the varying influence of pH, temperature, protein and detergent concentration, all of which influence a detergent's ability to expose enzyme activity trapped in vesicles (Jorgensen et al. 1971). As it is important to obtain the critical micelle concentration to achieve maximal activation it was decided that a more reliable method of exposing activity was achieved using the four freeze-thaw cycles just prior to analysis.

Caution must be taken if one wants to convert these activity measures to Na^+,K^+-ATPase concentration values, as there are uncertainties involved in determining the molecular activity of the enzyme (Kjeldsen et al. 1984a). It is also assumed that the number of molecules is the sole determinant of the maximal K^+-stimulated 3-*O*-MFPase activity. Calculations using previously established turnover rates from purified enzyme preparations (Kjeldsen et al. 1988) would be inappropriate under the modified conditions of the assay. With an increased rate of reaction new turnover rates appropriate for the modified conditions are required.

In conclusion, several important modifications were made to the K^+ stimulated 3-*O*-MFPase assay. The maximal activity was five-fold higher than other reports for human muscle, predominantly due to use of an increased substrate concentration. Variability was reduced by measuring 3-*O*-MFPase activity in a single run. This modified, sensitive assay allows for reliable and valid measurement of Na^+,K^+-ATPase activity in human skeletal muscle.

Chapter 5 Fatigue depresses maximal Na^+,K^+-ATPase activity in human skeletal muscle

5.0 Introduction

Muscle fatigue is a complex phenomenon involving multiple factors, with the importance of each factor dependent on the intensity and duration of exercise, muscle fibre composition and the individual degree of physical training (Fitts & Balog 1996; McKenna 1992). One postulated mechanism contributing to fatigue during intense exercise is an impairment of membrane excitability, caused by deterioration in transmembrane Na^+ and K^+ gradients, consequent to inadequate Na^+,K^+-ATPase activity (Clausen et al. 1987; Jones & Bigland-Ritchie 1986; Nielsen & Overgaard 1996; Overgaard et al. 1997; Verburg et al. 1999).

At rest the Na^+, K^+-ATPase enzyme is activated to only a few percent of its theoretical maximum rate (Clausen et al. 1987; Clausen & Everts 1989; Everts & Clausen 1994; Hazeyama & Sparks 1979). However, during contractions the Na^+, K^+-ATPase enzyme is rapidly activated, with the mechanism for activation being a combination of electrical, ionic, and hormonal factors (Clausen 1998; Green 1998; McKenna 1998). This activation constrains the excitation-induced rise in $[Na^+]_i$ and $[K^+]_e$ and thereby limits cellular depolarisation, thus acting to maintain muscle excitability. Despite this activation, Na^+ and K^+ gradients across the sarcolemma are not always maintained during muscle contractions induced by electrical stimulation (Clausen et al. 1987). This was clearly shown by Balog & Fitts (1996) who used ion-selective electrodes to study the effects of

fatiguing high frequency stimulation (150Hz) on $[Na^+]_i$ and $[K^+]_i$ in frog semitendinosus muscle. As a result of the fatiguing stimulation, $[Na^+]_i$ rose from 16mM to 49mM and $[K^+]_i$ fell from 142mM to 97mM. Consequently the membrane potential declined from –83mV to –70mV (Balog & Fitts 1996). These excitation-induced changes in ion gradients have been shown to contribute to the development of fatigue through rapid membrane depolarisation, reduced action potential amplitude, and slow inactivation of voltage dependant Na^+ channels (Hodgkin & Horowitz 1959; Juel 1988; Overgaard et al. 1999; Ruff 1996). An elevated extracellular $[K^+]$ can reduce excitability in skeletal muscle, with a 40% and 95% loss in contractile force evident when $[K^+]_e$ was elevated to 10mM and 12.5mM respectively (Cairns et al. 1995; Clausen et al. 1993; Juel 1988; Lännergran & Westerblad 1986). This problem is exacerbated when $[Na^+]_e$ is simultaneously reduced. Overgaard et al. (1999) showed that exposure to 85mM Na^+ and 9 mM K^+ decreased the M-wave area (compound action potential), by 54% and reduced tetanic force by 50% in isolated rat soleus muscle stimulated through the nerve.

The importance of active Na^+, K^+ transport in maintaining muscle excitability and force generation has been demonstrated by inducing acute Na^+, K^+-ATPase activation or deficiency. The acute loss of force in intact soleus muscle exposed to lowered $[Na^+]_e$ and increased $[K^+]_e$ could be substantially recovered by stimulating Na^+, K^+-ATPase activity with 10^{-5} M of the β_2 agonist salbutamol (Bouclin et al. 1995, Overgaard et al. 1997). The recovery of force was completely inhibited with 10^{-3} M ouabain (Overgaard et al. 1997). In studies where Na^+, K^+-ATPase content is acutely reduced by ouabain inhibition, force during repeated tetani is subsequently compromised (Clausen et al. 1993; Nielsen & Clausen 1996). In isolated rat soleus and extensor digitorum longus muscles, graded Na^+, K^+-ATPase inhibition with ouabain induced a marked reduction in force development (Nielsen & Clausen 1996). A 54% reduction in rat soleus muscle Na^+, K^+-

ATPase content induced by K^+ deficiency also caused a proportional loss in force (Nielsen & Clausen 1996). The resultant reduction in force with reductions in Na^+, K^+-ATPase content highlight the importance of Na^+, K^+-ATPase to skeletal muscle contractile performance.

Whilst resting Na^+, K^+-ATPase activity in skeletal muscle has been investigated in animal models (Hundal et al. 1994; Judah et al. 1962; Nørgaard et al. 1984b) and in humans (Benders et al. 1993; Chapter 4), the effects of fatiguing exercise on skeletal muscle Na^+, K^+-ATPase activity have not been directly investigated. Sarcoplasmic reticulum Ca^{2+} ATPase activity is depressed with fatigue in animal and human models, most likely due to a structural alteration in the enzyme (Booth et al. 1997; Byrd et al. 1989a,b; Li 1999). Given the structural homology of the α subunit of the Ca^{2+} ATPase and Na^+, K^+-ATPase enzymes (Inesi et al. 1997), it is possible that structural alterations may be induced in both ion pumps with fatigue (Booth et al. 1997). Although numerous studies have looked at K^+ regulation and Na^+, K^+-ATPase content in exercising humans (Green et al. 1993; Kjeldsen et al. 1990a,b; McKenna et al. 1993) these studies have been restricted to the plasma $[K^+]$ response and $[^3H]$-ouabain binding site content. No studies have investigated the possibility that an acute depression of maximal in vitro Na^+, K^+-ATPase activity may occur with fatigue. Therefore this study tested the hypothesis that skeletal muscle Na^+,K^+-ATPase activity would be depressed at fatigue.

5.1 Methods

Eight healthy untrained males volunteered for the study and gave informed consent as described (Chapter 3). All experimental procedures for exercise tests, muscle biopsies and blood sampling and analyses, and respiratory data analyses have been fully described

(Chapter 3) as have details and validation of the 3-*O*-MFPase assay (Chapter 4). Briefly, all subjects completed an incremental exercise test to fatigue and on a separate day completed a muscle fatigue test (MFT) comprising 50 maximal knee extensions. All subjects had a muscle biopsy taken from the vastus lateralis muscle at rest and immediately after completion of MFT. Muscle biopsy samples were analysed for 3-*O*-MFPase activity and [^{3}H]-ouabain binding site content, fibre type proportions and metabolites. Arterialised-venous blood samples were taken from a dorsal hand vein before, during, and after the two exercise trials. Blood samples were analysed for blood [Hb], Hct, and plasma gases, [K^+], [H^+], and [lactate].

5.2 Results

5.2.1 Muscle fatigue test

Peak knee extensor muscle torque declined by 49.5 ±2.8 % during the 50 contractions on the Cybex dynamometer ($P < 0.05$, Figure 5.1). The cumulative work done was 10.68 ± 0.32 kJ.

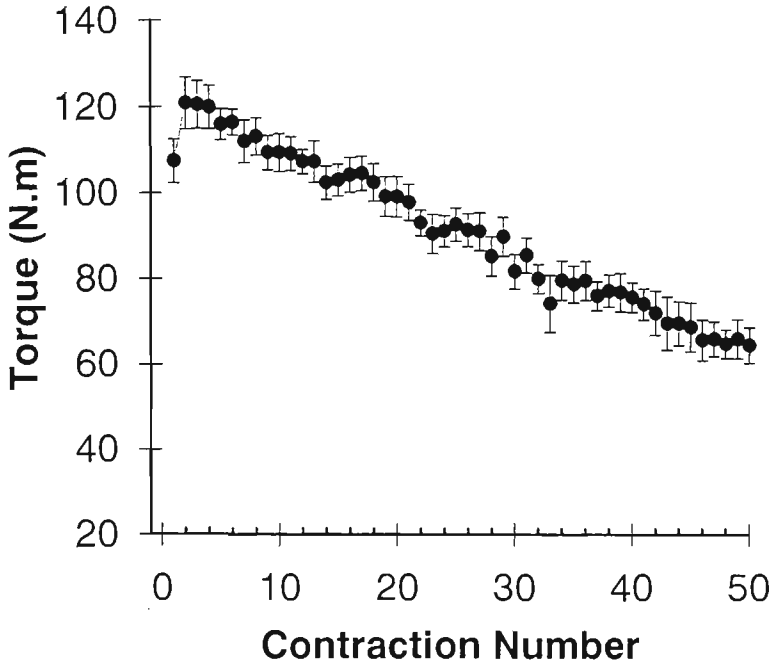

Figure 5.1 Knee extensor muscle peak torque during 50 maximal contractions on a Cybex isokinetic dynamometer. Mean ± SEM; n=8.

5.2.2 Fatigue effects on maximal 3-*O*-MFPase activity

The maximal *in-vitro* 3-*O*-MFPase activity in resting skeletal muscle was 207 ± 10 nmol min^{-1} g^{-1} wet wt. This was depressed by 13.8±4.1% ($P < 0.05$) to 179 ± 9 nmol min^{-1} g^{-1} wet wt at fatigue (Table 5.1). The depression with fatigue was similarly evident when activity was expressed relative to muscle protein content (-10.5±3.4%, $P < 0.05$, Figure 5.2, Table 5.1). (Note the 3-*O*-MFPase activity data for subject 3 was incomplete and was excluded from the table and future correlations with 3-*O*-MFPase activity). A suboptimal K^+ stimulating concentration of 5.5mM resulted in 3-*O*-MFPase activity that was approximately 75% of the maximal activity induced by 10mM [K^+]. When the 5.5mM KCl was used, the 3-*O*-MFPase activity in fatigued muscle was not significantly altered from rest (n=6, $P = 0.23$, Table 5.2).

Table 5.1 Maximal 3-*O*-MFPase activity (using 10mM KCl) in vastus lateralis muscle biopsied at rest and at fatigue in untrained subjects.

	3-*O*-MFPase activity (nmol min^{-1} g^{-1} wet wt.)		**3-*O*-MFPase activity (nmol min^{-1} g^{-1} protein)**	
Subject	**Rest**	**Fatigue**	**Rest**	**Fatigue**
1	227	202	1295	1123
2	224	177	1232	1057
4	167	130	951	790
5	203	191	1172	1127
6	234	179	1395	1148
7	219	198	1253	1159
8	170	177	1095	1002
Mean ±SEM n =7	207 ± 10	179 ± 9*	1199 ± 54	1058 ± 49*
Absolute decline (rest-fatigue)		27 ± 8		141 ± 26
% decline (rest-fatigue)		13 ± 4		12 ± 2

*fatigue < rest (P<0.05)

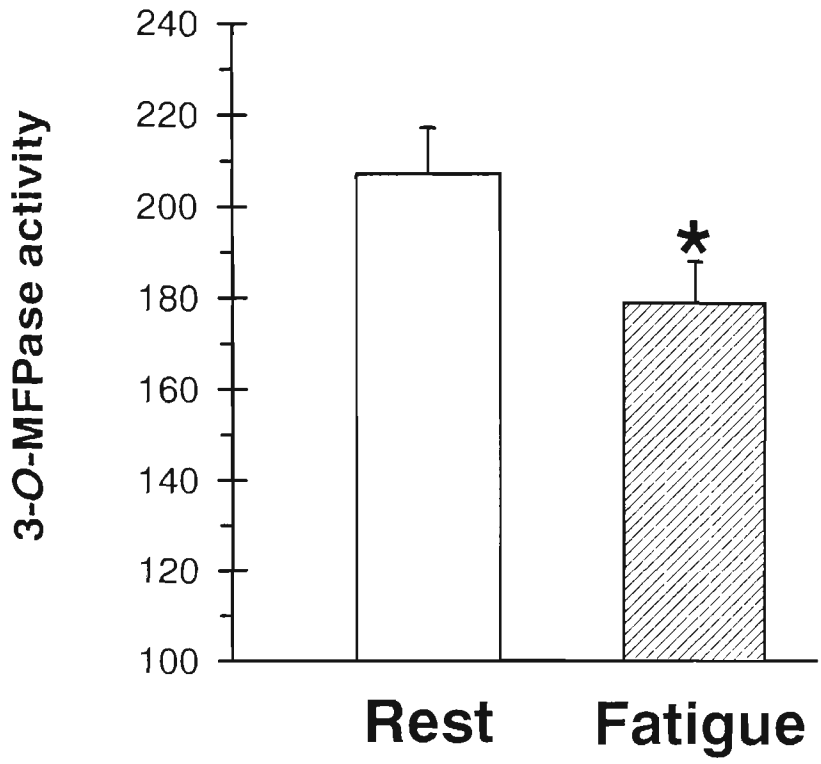

Figure 5.2 Maximal 3-*O*-MFPase activity (nmol min^{-1} g^{-1} wet wt.) from vastus lateralis muscle biopsied at rest and at fatigue expressed relative to muscle wet weight. Data are mean ± SEM; n=7; $^*P < 0.05$.

Table 5.2 Muscle 3-*O*-MFPase activity induced by a sub-optimal stimulating KCl concentration of 5.5mM, biopsied at rest and at fatigue in untrained subjects.

Subject	3-*O*-MFPase activity (nmol min^{-1} g^{-1} wet wt.)		3-*O*-MFPase activity (pmol min^{-1} mg^{-1} protein)	
	Rest	**Fatigue**	**Rest**	**Fatigue**
1	201	148	1144	824
2	152	133	833	794
4	133	80	756	483
5	141	128	811	754
6	153	159	912	1021
8	133	159	855	902
Mean ±SEM n= 6	152 ± 10	135 ± 12	885 ± 56	796 ± 74
% of maximal activity	73%	75%	72%	75%
Absolute decline (rest-fatigue)		27 ± 8		141 ± 26
% decline (rest-fatigue)		13 ± 4		12 ± 2

5.2.3 Muscle Na^+, K^+-ATPase content

The total Na^+, K^+-ATPase content in resting muscle for the 8 untrained subjects measured by ^3H ouabain binding was 306 ± 13 pmol.g^{-1} wet wt (Table 5.3).

Table 5.3 Na^+, K^+-ATPase content in vastus lateralis muscle biopsied at rest in untrained subjects.

Subject	Na^+,K^+-ATPase content ($pmol.g^{-1}$ wet wt)
1	329
2	375
3	305
4	329
5	250
6	275
7	285
8	302
Mean ±SEM	306 ± 13

5.2.4 Muscle fibre type

The untrained subjects had 52.7 ± 3.4% type I and 47.3 ± 3.4% type II muscle fibres.

5.2.5 Fatigue effects on muscle metabolites

There was a decrease in muscle ATP (13.5%), PCr (62%), and glycogen contents (24%) with fatigue, whilst muscle $[H^+]$ (>2-fold) lactate (11-fold), IMP (40-fold) and creatine contents (> 2-fold) all increased with fatigue ($P < 0.05$, Table 5.4).

Table 5.4 Muscle substrate and metabolite contents in vastus lateralis muscle biopsied at rest and at fatigue in untrained subjects.

	Rest	Fatigue
ATP	25.67 ± 0.85	19.51 ± 1.71*
ADP	2.77 ± 0.18	2.90 ± 0.47
AMP	0.13 ± 0.02	0.15 ± 0.02
IMP	0.08 ± 0.01	3.26 ± 0.84*
PCr	93.3 ± 2.3	35.8 ± 3.5*
Cr	42.0 ± 2.6	99.5 ± 4.6*
Lactate	8.0 ± 0.8	91.6 ± 10.0*
$[H^+]$	68.4 ± 2.7	152.6 ± 13.3*
Glycogen	469 ± 34	356 ± 31*

Values are expressed as mmol.kg^{-1} dry weight of muscle, except H^+ (nmol.L^{-1}), and glycogen (mmol glucosyl units.kg^{-1} dry muscle), n=8, Mean ± SEM. * Rest different to fatigue, $P < 0.05$.

5.2.5.1 Relationships between muscle 3-*O*-MFPase activity and muscle metabolites at rest and fatigue

To determine whether the in vitro muscle 3-*O*-MFPase activity may be related to metabolic status the relationships between muscle 3-*O*-MFPase activity, Na^+, K^+-ATPase content, muscle function and fibre type were determined. There was a significant positive relationship between 3-*O*-MFPase activity and muscle PCr contents in resting skeletal muscle (Fig 5.3A). In fatigued muscle there was a significant inverse relationship between muscle $[H^+]$ and 3-*O*-MFPase activity (Fig 5.3B), but this was largely dependent

on a single subject who displayed a high [H^+] and low 3-*O*-MFPase activity at fatigue. There were no other significant relationships between 3-*O*-MFPase activity and metabolites in resting or fatigued muscle.

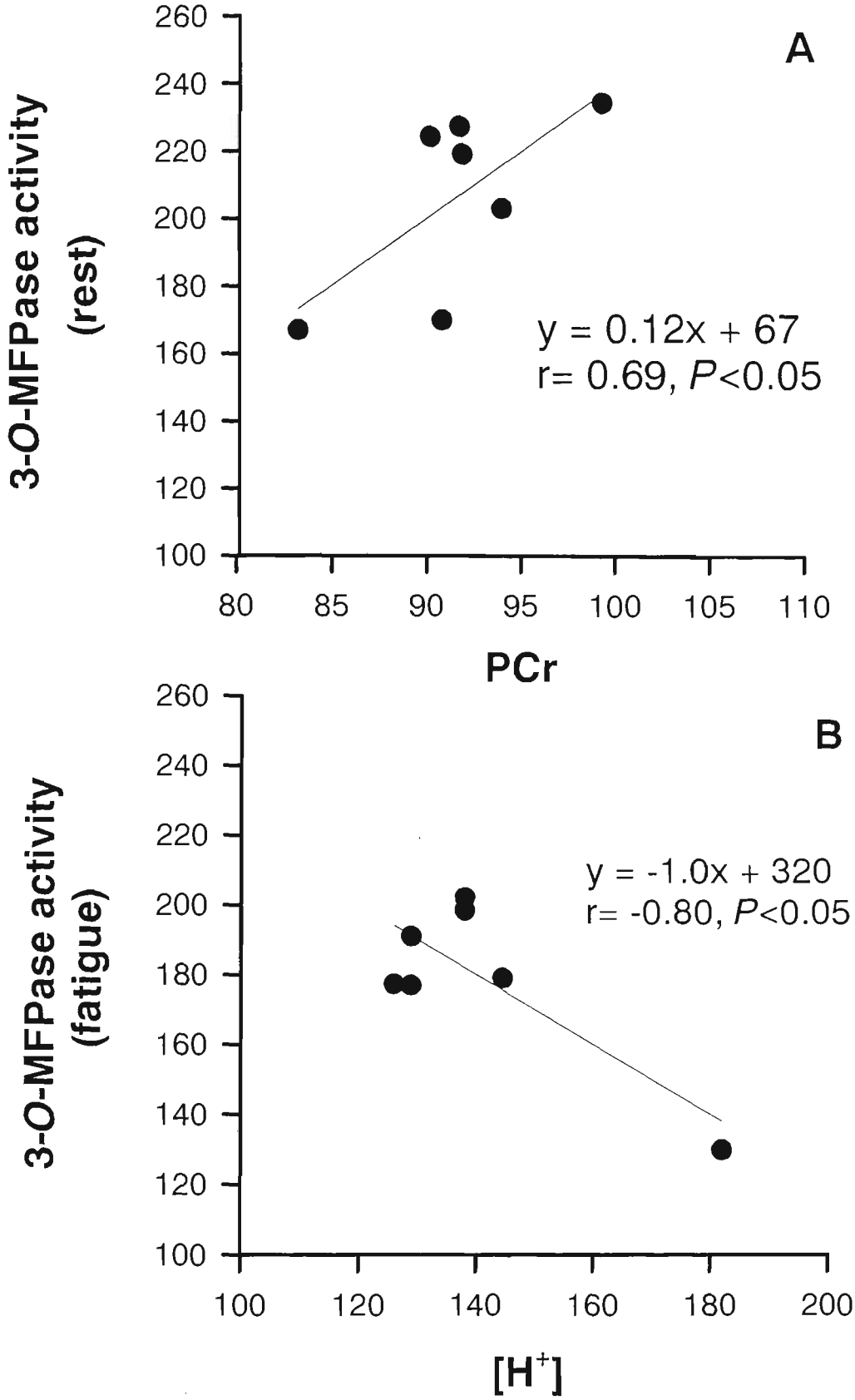

Figure 5.3 The relationships between (A) resting muscle maximal *in vitro* 3-*O*-MFPase activity (nmol min^{-1} g^{-1} wet wt) and PCr content, n=7, and (B) fatigued muscle maximal *in vitro* 3-*O*-MFPase activity (nmol min^{-1} g^{-1} wet wt) and fatigued muscle $[H^+]$, n=7.

When the rest and fatigue data were pooled, there was a moderate relationship between 3-*O*-MFPase activity and both ATP and PCr contents ($P < 0.05$, Fig 5.4 A,B) and an inverse relationship with lactate ($P < 0.05$, Fig 5.4 C). However, there were no significant relationships between the change in activity (rest –fatigue) and either the absolute or percentage changes in metabolites.

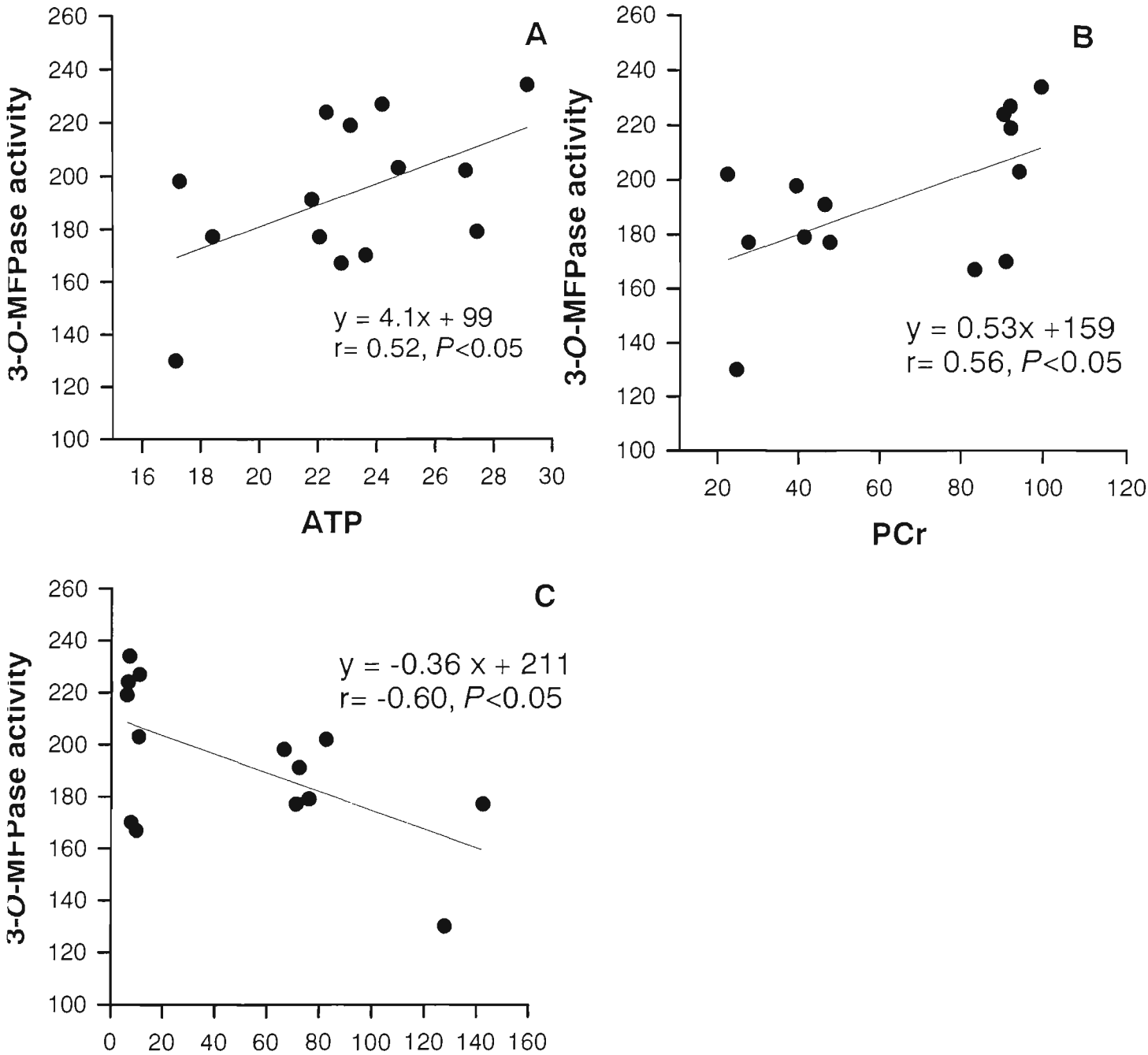

Figure 5.4 The relationships between maximal *in vitro* 3-*O*-MFPase activity (nmol.min^{-1}.g^{-1} wet wt) and pooled (rest and fatigue) muscle ATP (**A**), PCr (**B**) and (**C**) lactate contents (mmol.kg^{-1} ,dry wt) n=7.

5.2.5.2 Relationships between 3-*O*-MFPase activity, Na^+, K^+-ATPase content, muscle fibre type and muscle performance

There were no significant relationships between 3-*O*-MFPase activity, Na^+, K^+-ATPase content, muscle fibre type or indices of muscle performance including fatigue index or total work.

5.2.6 Fluid and electrolytes changes during the MFT

5.2.6.1 Plasma volume changes during the MFT

Plasma volume decreased ($P < 0.05$) by 5.7 ± 1.3% during fatiguing exercise and for 5 min following exercise, then did not differ significantly from resting levels thereafter (Figure 5.5A).

5.2.6.2 Plasma [lactate] and [H^+]

Arterialised-venous plasma [H^+] rose slightly from 37.5 ± 0.5 to 39.4 ± 0.6 nmol.l^{-1} at the end of exercise, peaked at 5 min post and returned to resting levels by 20 min post-exercise (Figure 5.5B). Plasma [lactate] rose three-fold during MFT, peaked 2 min into recovery and slowly declined until not differing from resting levels by 20 min post (Figure 5.5C).

5.2.6.3 Plasma [K^+] during MFT

Arterialised-venous plasma [K^+] increased from 3.89 ± 0.07 mM at rest to 4.81 ± 0.17 mM at peak exercise (Figure 5.5D). The $\Delta[K^+]$ at peak exercise was 0.92 ± 0.13 mM and the $\Delta[K^+]$ expressed relative to work done was 85.7 ± 13.0 nmol.l^{-1}.J^{-1}. The rate of decline in [K^+] from peak exercise to 1 and 2 min recovery was 0.42 ± 0.10 and 0.35 ± 0.07 mmol.l^{-1}.min^{-1}, respectively.

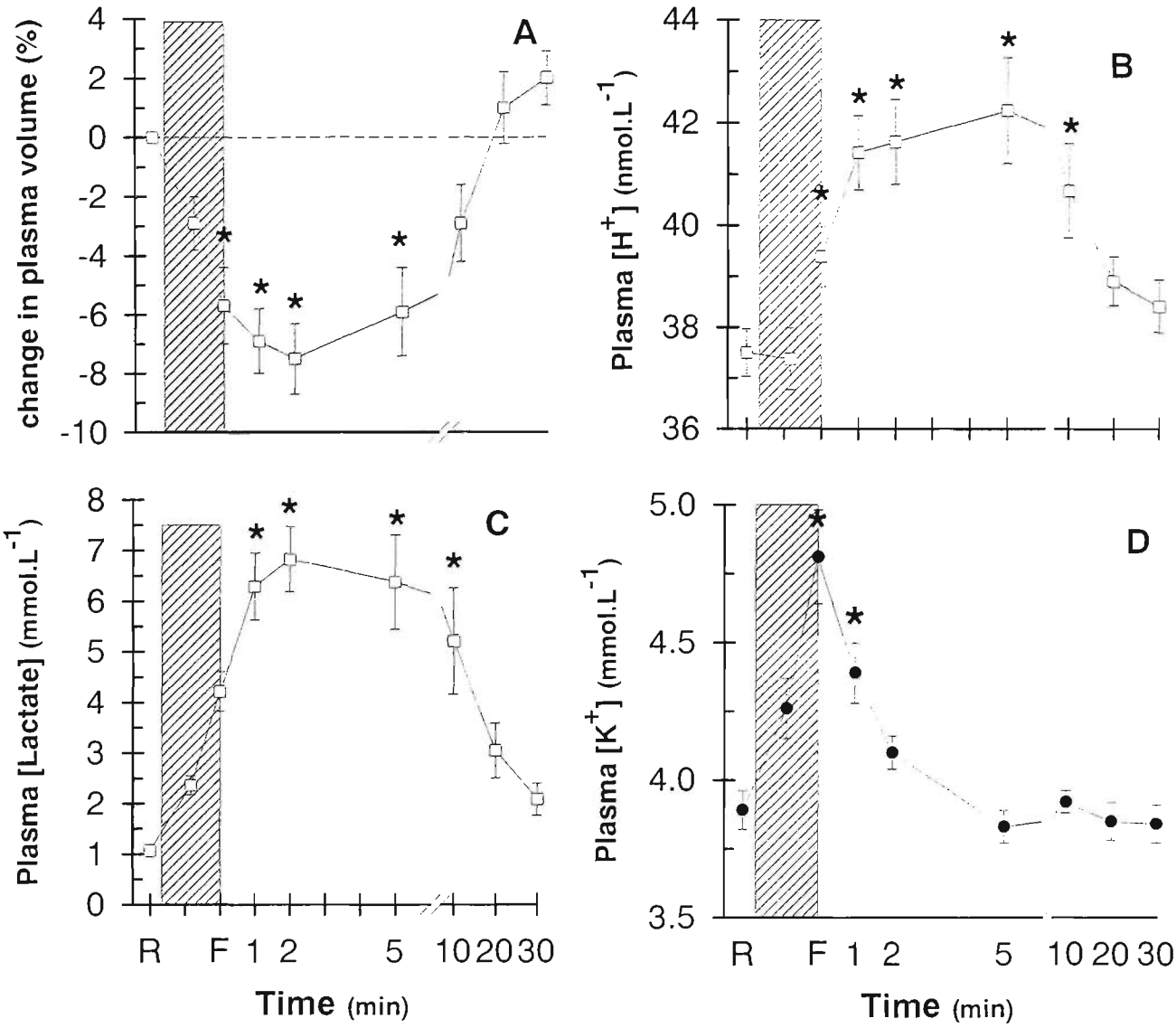

Figure 5.5 Arterialised venous (**A**) ΔPlasma volume, (**B**) plasma [H^+], (**C**) plasma [Lactate], and (**D**) plasma [K^+] at rest (R) and at fatigue (F) during the MFT (denoted by hatched bar) and 30 min of recovery. * different from rest, $P < 0.05$. Data: n=8, Mean ± SEM.

5.2.7 Relationships between 3-*O*-MFPase activity, Na^+, K^+-ATPase content, muscle performance and K^+ response during MFT

To determine whether the absolute content or activity of Na^+, K^+-ATPase might determine the plasma $[K^+]$ response, the relationships between 3-*O*-MFPase activity, Na^+, K^+-ATPase content, muscle performance and K^+ response during MFT were examined. Resting skeletal muscle 3-*O*-MFPase activity was not related to peak plasma $[K^+]$, but there was a significant inverse relationship with $\Delta[K^+]$ and $\Delta[K^+].work^{-1}$ ratio during the MFT (Fig 5.6A, B). Surprisingly, however, an inverse relationship was also found between 3-*O*-MFPase activity and fall in arterialise-venous plasma $[K^+]$ at 1 and 2 min post exercise during the MFT (Fig 5.6 C,D).

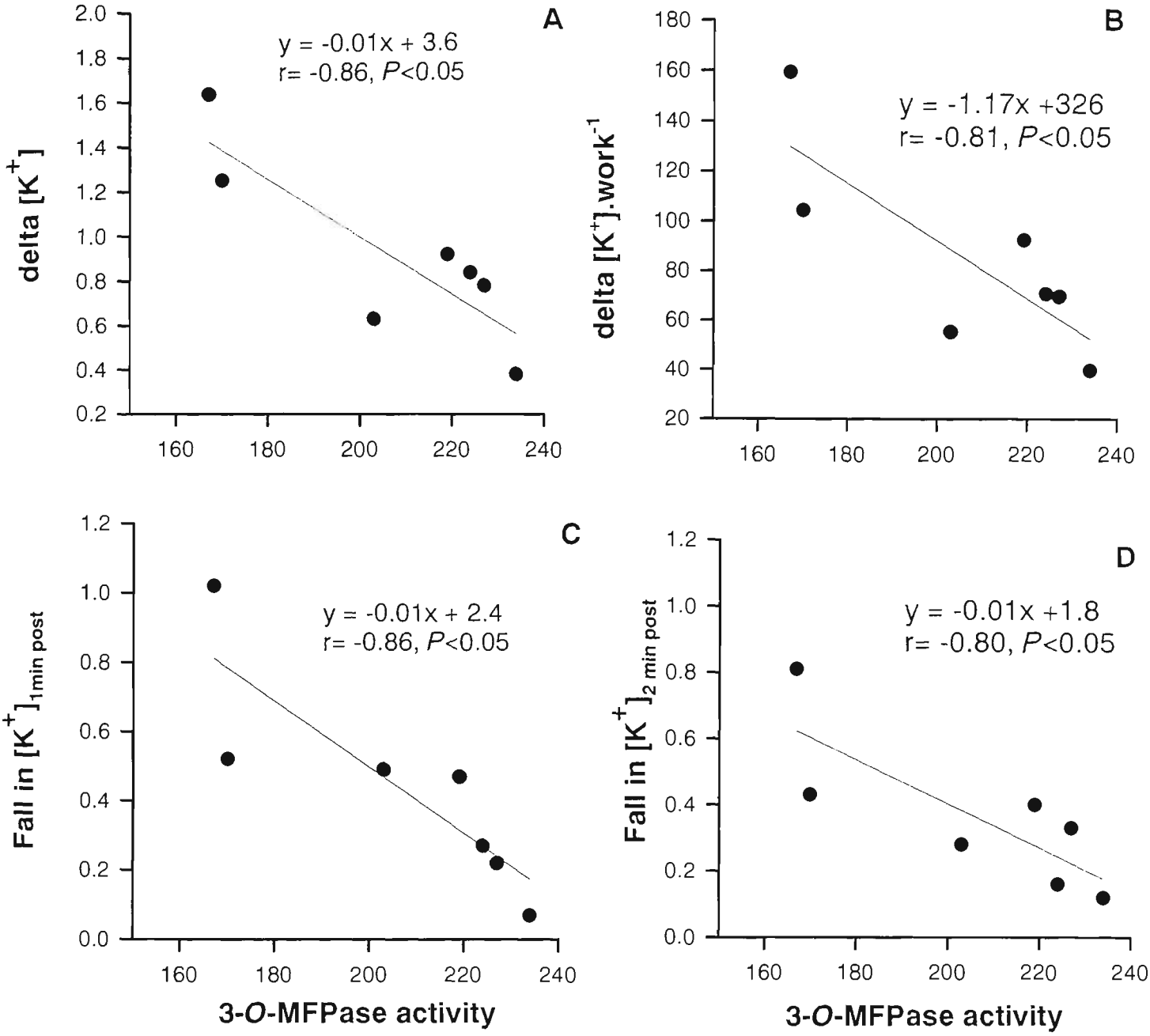

Figure 5.6 The relationship between maximal *in vitro* 3-*O*-MFPase activity in resting muscle (nmol min^{-1} g^{-1} wet wt) and (**A**) $\Delta[K^+]$, (mM) (**B**) $\Delta[K^+].work^{-1}$ ratio ($nmol.l^{-1}.J^{-1}$) (**C**) Fall in $[K^+]$ at 1 min post and (**D**) Fall in $[K^+]$ at 2 min post for the MFT. n=7.

5.2.8 Cardiorespiratory measures during the incremental exercise test

The work output results and cardiorespiratory measures taken during the incremental exercise test are summarised in Table 5.5.

Table 5.5 Peak incremental exercise test cardiorespiratory and work output data for the untrained subjects. Mean ± SEM, n = 8.

Peak variable	
$\dot{V}O_2$ ($l.min^{-1}$)	3.55 ± 0.40
$\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$)	44.4 ± 1.8
$\dot{V}_E$ ($l.min^{-1}$)	167.0 ± 7.0
HR ($b.min^{-1}$)	190 ± 2
Workrate (W)	313 ± 11
Total work (kJ)	128 ± 8

5.2.8.1 Plasma volume and plasma [K^+] during incremental exercise test

Plasma volume decreased ($P < 0.05$) by 13.4% during the incremental exercise, then returned to resting levels by 30 min recovery (Figure 5.7A). Arterialised-venous plasma [K^+] increased from 3.95 ± 0.06 mM at rest to 6.14 ± 0.17 mM at peak exercise ($P < 0.05$) and did not differ from resting levels from 5 min post-exercise (Figure 5.7B). The $\Delta[K^+]$ at peak exercise was 2.19 ± 0.16 mM and the $\Delta[K^+]$ expressed relative to work done was 18.5 ± 2.3 $nmol.l^{-1}.J^{-1}$. The rate of decline in [K^+] from peak exercise to 1 min and to 2 min recovery was 0.52 ± 0.09 and 0.69 ± 0.06 $mmol.l^{-1}.min^{-1}$, respectively.

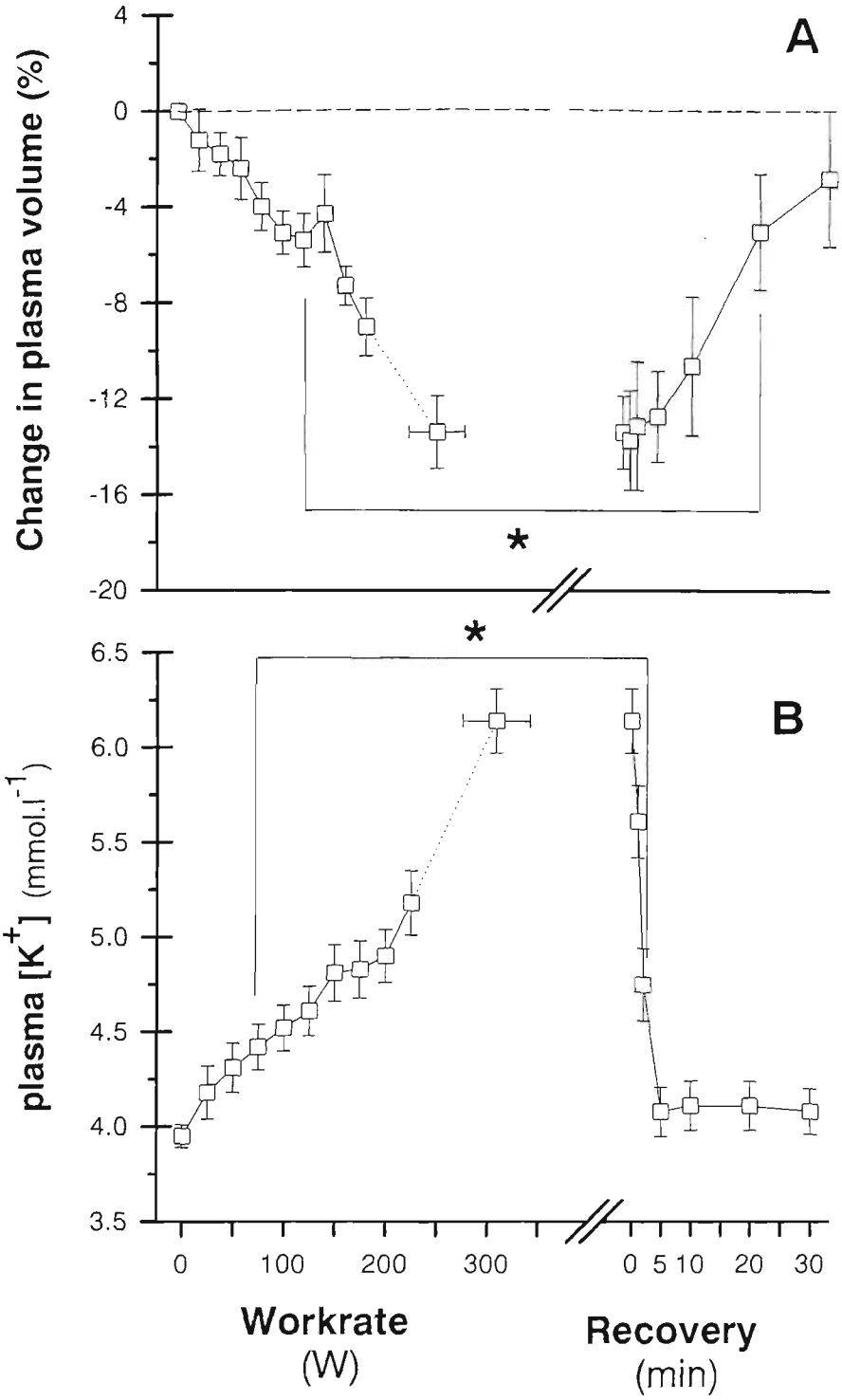

Figure 5.7 (**A**) Decline in arterialised venous plasma volume and (**B**) Plasma [K^+] at rest, during incremental exercise and 30 min of recovery. Data: n=8, Mean ± SEM. *significantly different from rest ($P < 0.05$), dashed line indicates zero change in plasma volume.

5.2.9 Relationship between 3-*O*-MFPase activity, Na^+, K^+-ATPase content, and plasma [K^+] during the incremental exercise test

There was a tendency for an inverse relationship between 3-*O*-MFPase activity (wet wt) in resting muscle and $\Delta[K^+]$ (Fig 5.8A), but this was significant when $\Delta[K^+]$ was expressed relative to work performed (Fig 5.8B) in the incremental test. There was no significant relationship between 3-*O*-MFPase activity and fall in $[K^+]$ 1 and 2 min post incremental exercise (Fig 5.8C,D).

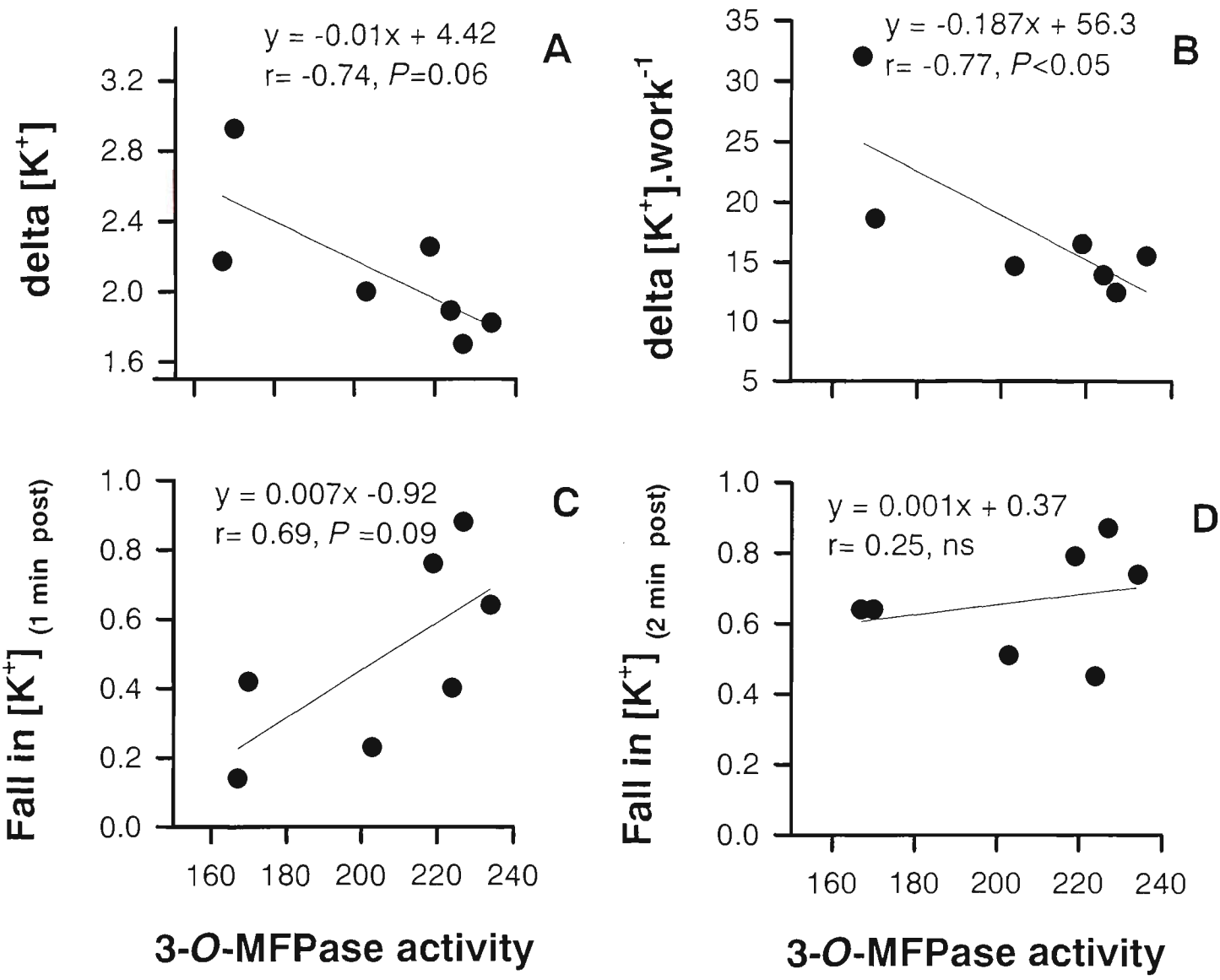

Figure 5.8 The relationship between maximal *in vitro* 3-*O*-MFPase activity at rest (nmol min^{-1} g^{-1} wet wt) and (**A**) $\Delta[K^+]$, (mM) (**B**) $\Delta[K^+].work^{-1}$ ratio (nmol.l^{-1}.J^{-1}) (**C**) Fall in $[K^+]$ at 1 min post and (**D**) Fall in $[K^+]$ at 2 min post incremental exercise.

5.3 Discussion

5.3.1 Depression in Na^+, K^+-ATPase activity after intense fatiguing exercise

The most important finding from this study was the 13.8±4.1% decrease in maximal K^+ stimulated 3-*O*-MFPase activity in skeletal muscle with fatiguing exercise. This is the

first time this has been demonstrated and implicates Na^+, K^+-ATPase activity as another potential site for muscle fatigue during high intensity exercise in humans.

5.3.2 Critique of method used to measure Na^+, K^+-ATPase activity

As outlined in Chapter 4, the *in vitro* 3-*O*-methlyfluorescein phosphatase assay used to measure Na^+, K^+-ATPase activity was optimised for human skeletal muscle homogenates. There are no other established methods to directly measure Na^+, K^+-ATPase activity in human skeletal muscle, and the *in vitro* 3-*O*-MFPase assay is the most sensitive assay currently used to indirectly measure Na^+, K^+-ATPase activity. The specificity of the assay was confirmed with ouabain inhibition. Despite these positives, the assay does have several limitations. The first is that any acute *in vivo* effects on the Na^+, K^+-ATPase activity is unlikely to be detected. These might include increases in muscle temperature, $[H^+]$ and changes in metabolites that are not detectable in the standardised conditions of this *in vitro* 3-*O*-MFPase assay. In addition, the activating effects of various hormones, and electrical events are clearly not measurable with this *in vitro* assay. Secondly the measurement of maximal activity may not be physiologically relevant since it has been suggested that the Na^+, K^+-ATPase enzyme is only activated in the range of 15-50% of its theoretical maximum in human muscle (Hallén et al. 1994; Sejersted & Hallén 1987). Many studies have shown that the Na^+, K^+-ATPase is activated during muscle contraction (Clausen 1998; Clausen & Everts, 1989; Everts & Clausen 1994; Everts et al. 1988; Hazeyama & Sparks 1979) but the exact extent of activation in human skeletal muscle is not known. The results of this study showing a depression in maximal *in vitro* Na^+, K^+-ATPase activity with fatigue suggest a reduced reserve in Na^+, K^+-ATPase activity. The physiological significance of this reduced reserve is not clear from this study and remains to be elucidated.

Finally the maximal K^+-stimulated phosphatase activity represents the terminal phosphatase reaction in the process of ATP cleavage, Na^+ extrusion, and K^+ reaccumulation in the Na^+, K^+-ATPase cycle (Elmosehli et al. 1994). Thus the assay does not indicate the physiological activity *in vivo* in comparison to the *in vitro* maximal K^+-stimulated phosphatase activity. It is possible therefore that this assay does not reflect the maximal activity of the complete Na^+, K^+-ATPase cycle. Despite these limitations a clear effect of fatigue has been shown on the maximal K^+ stimulated 3-*O*-MFPase assay indicating a compromised reserve in Na^+, K^+-ATPase activity.

5.3.3 Mechanisms for fatigue induced depression in Na^+, K^+-ATPase activity

The fatigue-induced depression in Na^+, K^+-ATPase activity was evident when the data were expressed relative to wet weight and relative to muscle protein content. This indicates that the lower activity at fatigue was not due to fluid shifts into muscle and therefore diluted Na^+, K^+ ATPase in the fatigued muscle. This, combined with the fact that the incubation medium was the same for rest and fatigue muscle, it is most likely that a structural alteration was the cause of the fatigue-induced depression in the *in-vitro* Na^+, K^+-ATPase activity. The exact mechanisms for the proposed structural alterations to the Na^+, K^+-ATPase enzyme are unclear and cannot be resolved from this study. Several possibilities include accumulation of Ca^{2+}, reactive oxygen species, metabolic perturbations, and increased temperature. Due to structural homology of the catalytic subunits of the Na^+, K^+-ATPase and Ca^{2+} ATPase (Inesi et al. 1997), factors that affect Ca^{2+} ATPase may also affect Na^+, K^+-ATPase activity. Interestingly, Ca^{2+} ATPase activity is decreased in human muscle during both prolonged (Booth et al. 1997) and brief contractions (Li 1999). Structural alterations suggested to occur in Ca^{2+} ATPase with fatigue (Booth et al. 1997), has been proposed to be due to increased oxygen free radicals

(Davies et al. 1992), exercise-induced calcium activated proteases (Belcastro et al. 1993), or alterations (Gilchrist et al. 1992). No studies have investigated the effects of fatigue on Na^+, K^+-ATPase in relation to structural alterations, ROS or temperature changes. A possible explanation for the decreased Na^+, K^+-ATPase activity is that structural alterations may decrease K^+ affinity.

5.3.3.1 Metabolic Effects

During the MFT, significant associations were seen between Na^+, K^+-ATPase activity and the content of ATP and PCr, and muscle lactate using both rest and fatigue muscle. However this does not imply a causal relationship, rather it is possible that these marked metabolic changes reflect parallel processes occurring with fatigue. Any causal relationship between these metabolic perturbations and the depressed *in vitro* Na^+, K^+-ATPase activity would need to be the result of *in-vivo* structural changes that persisted when measuring the *in vitro* maximal Na^+, K^+-ATPase activity. Metabolic perturbations may be exacerbated in the *in vivo* situation and could be additional to structural effects on the Na^+, K^+-ATPase. It is also important to clarify that the metabolite measurements were global measures and do not reflect localised changes around the Na^+, K^+-ATPase where some metabolic compartmentalisation has been proposed (James et al. 1996).

A link between glycolysis and Na^+, K^+-ATPase activity has been demonstrated in rat skeletal muscle (James et al. 1996, 1999). Using monensin (Na^+ ionophore) to raise intracellular [Na^+] in isolated rat skeletal muscle, James et al. (1996) showed an ouabain-suppressible (60%) stimulation of glycolysis and increase in lactate production under fully aerobic conditions. The authors suggested that removal of glucose from their incubation medium would inhibit Na^+, K^+-ATPase activity. James et al. (1996) also implied that a significant fraction of the ATP to fuel the Na^+, K^+-ATPase must be supplied

by glycolysis, and that a high degree of metabolic compartmentalisation occurs. Thus the fuel for Na^+, K^+-ATPase activity seems to derive from glycolysis and any local changes in fuel supply may well compromise Na^+, K^+-ATPase activity *in vivo*, but this may not be detected in global cellular measures of metabolites or muscle homogenate Na^+, K^+-ATPase activity measures.

If the depressive effect of fatigue on Na^+, K^+-ATPase was exacerbated in the *in vivo* situation where energy supply and pH are likely to be compromised, then its physiological importance in the etiology of muscle fatigue is paramount. Na^+, K^+-ATPase activity, when assessed via the 3-*O*-MFPase activity, has a broad pH range of 7.0-8.0. However the inhibition of Na^+, K^+-ATPase activity (assessed through P*i* production) increases as pH falls below 7.0 (Huang & Askari 1984). The pH of the fatigued muscle in this study was 6.82 indicating that Na^+, K^+-ATPase activity may be further compromised, adding to any structural alterations that may have occurred. The compartmentalisation theory that the Na^+, K^+-ATPase uses glycolysis to provide ATP (James et al 1996) may invoke a local pH drop due to the build up of lactic acid. The depressive effect of low muscle pH on Na^+, K^+-ATPase activity is amplified by increases in intracellular P*i*, which increases in fatigued muscle (Dawson et al. 1988; Huang & Askari 1984; Weiner et al. 1990). The inhibition of the Na^+, K^+-ATPase by P*i* (half maximal inhibition at 2-3mM) occurs when the P*i* is covalently incorporated into the same aspartyl residue of the enzyme that is phosphorylated in the course of ATP hydrolysis (Huang & Askari 1984; Mercer & Dunham 1981).

5.3.3.2 Reactive Oxygen Species

One possible mechanism underlying the depressed maximal Na^+, K^+-ATPase activity found in fatigued muscle is an elevation of reactive oxygen species (ROS) in muscle. Resting skeletal muscle produces free radicals and their production greatly increases during contraction (Davies et al. 1982, Reid et al. 1992). The Na^+, K^+-ATPase enzyme activity is dependent upon an intact phospholipid bilayer and any modifications to the phospholipid moiety could modify the protein's function. Impairment of sarcolemmal membrane integrity due to the effect of free radicals may occur, similar to lipid peroxidation observed in the SR membrane (Davies et al. 1982). The effect of ROS on skeletal muscle Na^+, K^+-ATPase has not been extensively studied (Kourie 1998). I am only aware of one study to have investigated ROS effects on Na^+, K^+-ATPase in skeletal muscle (abstract, Clough 1996). Clough (1996) reported a 70% decline in Na^+, K^+-ATPase activity in rat rectus femoris muscle when exposed to the free radical generating system of t-butylhydroperoxide and horseradish peroxidase. This inhibition of Na^+, K^+-ATPase activity in skeletal muscle probably involves disruption of the lipid bilayer or oxidation of SH groups on the Na^+, K^+-ATPase, but the precise mechanism remains unresolved. However it is known that myocardial Na^+, K^+-ATPase is susceptible to ROS-induced lipid peroxidation. Under ischaemic conditions, a 3-fold increase in malondialdehyde resulted in a 16% reduction in ouabain sensitive Na^+,K^+-ATPase activity, as calculated from P*i* production (Kim & Akera 1987). In pig coronary artery, the Na^+, K^+-ATPase hydrolytic activity and transport of Rb^+ were uncoupled upon exposure to peroxide or superoxide (Elmoselhi et al. 1994).

It has been suggested that ROS have a significant adverse impact on a number of ATPases, especially those bearing a sulphydryl (SH) group which are susceptible to ROS (Boldyrev et al. 1997; Körge 1998). The extent to which ROS-induced disruption to

sarcolemmal membrane proteins such as Na^+, K^+-ATPase occurs and whether this contributes to skeletal muscle fatigue remains to be determined.

5.3.3.3 Ca^{2+} inactivation of Na^+, K^+-ATPase activity

Another possible mechanism for the depressed Na^+, K^+-ATPase activity with fatigue is a rise in intracellular [Ca^{2+}] (Breier et al. 1998; Sulová et al. 1998). Whilst the Ca^{2+} transient during contractions actually decreases with fatigue (Westerblad et al. 1993), there is a progressive rise in the resting intracellular [Ca^{2+}] (Gissel & Clausen 1999). In millimolar concentrations Ca^{2+} inhibits both the Na^+, K^+-ATPase hydrolytic activity (Huang & Askari 1982; Yingst et al. 1992) and transport activity of the enzyme (Stankovicová et al. 1995). The inhibitory concentrations of Ca^{2+} can be shifted from millimolar to micromolar levels by modulation with calmodulin or calnactin (Sulova et al. 1998). Okafor et al. (1997) showed that calmodulin-dependant phospholipase A_2 is involved in Ca^{2+}-induced inhibition of the Na^+, K^+-ATPase. Gissel and Clausen (1999) showed that chronic low frequency stimulation led to increased sarcolemmal Ca^{2+} uptake, and this increased cytoplasmic [Ca^{2+}] has been attributed to various cellar damage pathways (Duan et al. 1990, McCutcheon et al. 1990). Increased intracellular [Ca^{2+}] has been shown to decrease the hydrolytic activity, as well as the transport (and/or electrogenic) activities of the Na^+, K^+-ATPase in resting muscle (Huang & Askari 1982; Stankovicová et al. 1995) and human red blood cells (Yingst et al. 1992). Thus it is possible that fatigue will induce a rise in intracellular [Ca^{2+}] and this may induce a conformational change in the Na^+, K^+-ATPase enzyme, and thereby reduce its activity and impair muscle function. A localised increase in cytoplasmic Ca^{2+} may stop further release from the SR (Lamb et al. 1995) leading to excitation-contraction uncoupling. This uncoupling may in addition involve inhibition of the Na^+, K^+-ATPase, protecting

against further contraction and deleterious increases in intracellular [Ca^{2+}]. Ca^{2+} induced inactivation of the Na^+, K^+-ATPase may be an important mediator in a coordinated downregulation of contractile activity.

Factors that temporarily or permanently alter the structure of the Na^+, K^+-ATPase are likely to cause the observed depression in maximal *in vitro* Na^+, K^+-ATPase activity. More than likely Ca^{2+} and ROS act additively or synergistically to compromise Na^+, K^+-ATPase activity and/or reduce K^+ sensitivity of the enzyme as they have both been shown to affect Na^+, K^+-ATPase function (Huang & Askari 1982; Clough 1996; Stankovicová et al. 1995; Yingst et al. 1992). Whether it is allosteric modification, altered lipoprotein interaction or a conformational change that comprises this depressed Na^+, K^+-ATPase activity cannot be determined from this study, but certainly warrants further investigation. The metabolic changes that occurred during the fatiguing exercise are also unlikely to alter the structure of the Na^+, K^+-ATPase but these perturbations may act in addition to the structural changes to further reduce Na^+, K^+-ATPase activity *in vivo*.

5.3.4 Relationship between Na^+, K^+-ATPase activity, content, and muscle performance

There was no significant relationship between Na^+, K^+-ATPase activity and Na^+, K^+-ATPase content. This finding is surprising given that activity should be largely determined by Na^+, K^+-ATPase content. However, the relatively small sample size could skew results. In addition, each Na^+, K^+-ATPase activity measure has a variability of around 5% and ^3H ouabain binding also around 5-10%, which increases the scatter of the data. This relationship is further examined with larger sample sizes and trained subjects in the next chapter.

The relationship between Na^+, K^+-ATPase content or activity and muscle force is of interest, given that a 50% decline in Na^+, K^+-ATPase content induced by K^+ deficiency showed a proportional loss in force (Nielsen & Clausen 1996). However no relationships were evident between Na^+, K^+-ATPase activity or content and indices of muscle performance during the muscle fatigue test. Part of the reason for discrepancy between present findings and those of Nielsen & Clausen (1996) is that the latter induced a 50% decrease in functional Na^+, K^+-ATPase which was 4-fold greater than the 13.5% decline in Na^+, K^+-ATPase shown here. It may be that there is a threshold level of inactivation of the Na^+, K^+-ATPase required to significantly reduce muscle force. Our measure of 13.5% decline in Na^+, K^+-ATPase may represent some fibres with various gradations of activity loss from normal activity to complete inhibition. More severe exercise may lead to a greater loss of activity in more fibres, therefore a greater reduction in force.

Previous research has also investigated Na^+, K^+-ATPase content and indices of muscle performance in relation to training. Klitgaard & Clausen (1989) showed no correlation between Na^+, K^+-ATPase content and knee extensor exercise endurance but did show a weak correlation with maximal isometric force in elderly trained subjects. Madsen et al. (1994) also found no relationship between Na^+, K^+-ATPase content and time to fatigue in endurance trained subjects. In contrast to the findings of Evertsen et al. (1997) and Green et al. (1999a), this study showed no relationship between Na^+, K^+-ATPase content and $\dot{V}O_2$ peak. This is probably due to the small sample size and the group homogeneity with respect to $\dot{V}O_2$ peak in the present study. The present study was not a training study, therefore the purpose was not to induce an increase in $\dot{V}O_2$ peak as was the design of the Green et al. 1999a study. These relationships are further examined with larger sample sizes and trained subjects in the next chapter.

5.3.5 Relationships between Na^+,K^+-ATPase activity and plasma $[K^+]$ during exercise

Skeletal muscle Na^+, K^+-ATPase plays a vital role in plasma $[K^+]$ regulation in muscle and blood during exercise, and therefore the relationships between Na^+,K^+-ATPase activity and $[K^+]$ during exercise were examined. Inconsistent relationships were found between Na^+, K^+-ATPase activity and the $[K^+]$ response during both the MFT and incremental exercise test. This could partly be explained by the site of blood sampling. Blood was sampled from a warmed hand vein which simulated arterial blood as PO_2 values were above 80 mmHg (Appendix B). The increases in plasma $[K^+]$ during the MFT would have been much larger if samples could have been taken from a femoral vein draining the contracting muscle (Juel et al. 1990). Such studies have not been approved for use in our laboratory. There would also be clearance of K^+ from the blood through red blood cells and both inactive and contracting skeletal muscle Na^+,K^+-ATPase (Lindinger et al. 1999; Rolett et al. 1990). However Juel et al. (1999) recently showed that red blood cell $[K^+]$ during muscle activity was more likely to be due to water movements than a transmembrane flux of K^+. Activation of Na^+, K^+-ATPase will rapidly decrease venous $[K^+]$ (Hallén 1996; McKenna et al. 1997) but the decrease in $[K^+]$ in arterialised-venous blood will be less rapid and smaller in magnitude. Recently Juel et al. (2000b) has measured interstitial $[K^+]$ from contracting muscle during dynamic exercise, and this is the most appropriate site for determination of relationships between Na^+,K^+-ATPase activity and $[K^+]$ from exercising muscle, as raised interstitial $[K^+]$ fatigue at this site increases cell depolarisation and therefore contributes to fatigue. However, a significant inverse relationship was found between *in vitro* Na^+, K^+-ATPase activity and $\Delta[K^+]$ and $\Delta[K^+].work^{-1}$ during the MFT. Na^+, K^+-ATPase activity was related to the $\Delta[K^+].work^{-1}$ during the incremental exercise test. The incremental test induced a greater rise in $[K^+]$

and an increased activation of Na^+, K^+-ATPase compared to the MFT. However despite a twelve fold greater amount of work done, there was only a doubling of $\Delta[K^+]$, indicating much larger clearance of K^+ during the incremental test as well.

In light of these sample site limitations, combined with a small sample size, the relationships in this study between Na^+, K^+-ATPase activity and the plasma $[K^+]$ response during both the incremental and fatigue tests must be viewed with caution. However Schmidt et al. (1995) showed that a 9% reduction in functional Na^+,K^+-ATPase as a result of digoxin treatment resulted a doubling of arterio-venous $[K^+]$ difference in CHF patients. Further, supra-physiological stimulation frequencies that induce large fluxes of Na^+ and K^+ across the sarcolemma, have been shown to exceed the capacity of the Na^+, K^+-ATPase enzyme to counter the flow of these ions (Clausen and Nielsen 1994b). It appears that even a small reduction in functional Na^+, K^+-ATPase capacity may have important effects in countering exercise hyperkalemia. Opposed to this notion is the report that the Na^+, K^+-ATPase enzyme is only activated in the range of 15-50% of its theoretical maximum immediately (Hallen & Sejersted 1993). Therefore the maximal activity of the Na^+, K^+-ATPase is of less importance than the *in vivo* activation of the Na^+, K^+-ATPase during exercise.

5.4 Conclusions

This study confirms the hypothesis that a depression occurs in maximal *in vitro* Na^+, K^+-ATPase activity with intense exercise in human skeletal muscle. This is the first time this has been demonstrated in skeletal muscle. The mechanism(s) responsible for the fatigue-induced depression in Na^+, K^+-ATPase activity cannot be directly deduced from this

study, but most likely involve structural alterations in the Na^+, K^+-ATPase enzyme. These structural alterations may be due to ROS or increases in intracellular [Ca^{2+}]. It is possible that fatigue effects for *in vivo* Na^+, K^+-ATPase activity are even greater than the *in vitro* effects measured here, due to the combined effects of structural alterations and other metabolic and ionic perturbations.

The results of this study indicate a compromised Na^+, K^+-ATPase function at fatigue or at least a reduced reserve for Na^+, K^+-ATPase activation. Further work is required to determine the mechanism and likely impact of fatigue induced depression in Na^+, K^+-ATPase activity.

Chapter 6 Effect of Chronic Resistance Training and Endurance Training on Na^+,K^+-ATPase Function and Plasma $[K^+]$ During Exercise

6.0 Introduction

Although it is well established that endurance training induces an increase in muscle Na^+, K^+-ATPase content (see Table 2.3), relatively few studies have investigated the effect of resistance training on Na^+, K^+-ATPase content. Green et al. (1999a) showed that resistance training for 7 weeks induced a 16% rise Na^+, K^+-ATPase content. However this response to resistance training was inconsistent as this increase at 7 weeks did not persist with a further 5 weeks of training (Green et al. 1999a). Klitgaard & Clausen (1989) showed that elderly strength trained men had 45% higher Na^+, K^+-ATPase content that untrained elderly men, although this relative change is exacerbated by the inactivity in the older untrained men. Despite this, the relative changes are much higher than other training studies, which show a change of around 15% with endurance training (Evertsen et al. 1997; Madsen et al. 1994; McKenna et al. 1993). Therefore the effects of resistance training on Na^+, K^+-ATPase are variable and warrant further investigation. Of the studies that have investigated endurance training and resistance training effects on Na^+, K^+-ATPase content, none have investigated changes in both Na^+, K^+-ATPase activity and content. Therefore the first aim of this study was to examine both Na^+, K^+-ATPase content and activity in endurance trained and resistance trained subjects.

The previous chapter showed that acute fatiguing exercise decreased the maximal *in vitro* skeletal muscle Na^+, K^+-ATPase activity in untrained subjects. The training effects on this fatigue-induced depression have not been examined. It is known that both resistance

training and endurance training induce a fibre transition from IIB to IIA and therefore increase the fibre oxidative potential (Adams et al. 1993; Fitts 1996; Green et al. 1979; Kraemer et al. 1995). It is not known whether resistance training and endurance training will attenuate the fatigue-induced decline in Na^+, K^+-ATPase activity, and whether this will be influenced by the fibre type expression. Therefore the second aim of this study was to contrast the effects of fatigue on Na^+, K^+-ATPase activity in endurance trained, resistance trained and untrained muscle.

Training results in a blunting of the exercise-induced rise in plasma K^+ concentrations (Green et al. 1993, Harmer et al. 2000; Kjeldsen et al. 1990), while McKenna et al. (1993) showed that seven weeks of sprint training resulted in a decreased rise in plasma $[K^+]$ relative to work done. This reduced hyperkalemia during exercise has partly been attributed to an increased Na^+, K^+-ATPase content in skeletal muscle (Green et al. 1993). However no studies have related a reduction in hyperkalemia with a rise in Na^+, K^+-ATPase content or activity. There have been no reports of reduced hyperkalemia after resistance training. This study therefore explored the effects of chronic training on the relationship between Na^+, K^+-ATPase content, activity and the plasma $[K^+]$ during exercise.

Animal studies have shown a relationship between Na^+, K^+-ATPase content and muscle contractile performance (Clausen et al. 1993; Nielsen & Clausen 1996). The relationship between muscle function and Na^+, K^+-ATPase in humans is unclear. There was no correlation between Na^+, K^+-ATPase content and time to fatigue after isometric contractions (Klitgaard & Clausen 1989) or work in repeated sprints (McKenna et al. 1993). However there was a weak correlation between Na^+, K^+-ATPase content and

maximal isometric force (Klitgaard & Clausen 1989). Therefore there are inconsistencies in the relationships between Na^+, K^+-ATPase content, training, and performance and this requires further investigation.

This study therefore examined the influence of training status and fatigue on Na^+, K^+-ATPase activity in human skeletal muscle. The hypotheses tested were (1) Chronic resistance trained and endurance trained subjects will show an increased resting Na^+, K^+-ATPase activity and content compared to control subjects. (2) Chronic resistance trained and endurance trained subjects will be characterised by improved muscular performance, associated with a smaller fatigue-induced depression in Na^+, K^+-ATPase activity and a lower plasma $[K^+]$ response during exercise compared to control subjects.

6.1 Methods

Eight healthy untrained controls (UT, Chapter 5), eight resistance-trained subjects (RT) and eight endurance-trained subjects (ET) volunteered for the study and gave informed consent (Chapter 3). All experimental procedures for anthropometric tests, exercise tests, muscle biopsies and blood sampling and analytical procedures have been fully described earlier (Chapter 3 & 4). Briefly, all subjects completed anthropometric tests, a torque-velocity test and 50 maximal knee extensions on a Biodex isokinetic dynamometer. They also completed an incremental cycle ergometer exercise test with arterialised-venous blood samples taken from a dorsal hand vein before, during, and after the test. Blood samples were analysed for plasma acid-base status and gas tensions, $[K^+]$, [lactate], as well as [Hb] and Hct. On a separate day, subjects completed a muscle fatigue test (MFT) on a Cybex isokinetic dynamometer. All subjects had biopsies taken from the vastus lateralis muscle at rest and immediately after completion of the MFT. Resting muscle

biopsy samples were analysed for Na^+, K^+-ATPase activity and content, fibre type proportions and metabolites, while fatigue samples were analysed for Na^+, K^+-ATPase activity and metabolites. The same blood analyses for the incremental test were conducted for the MFT.

6.2 Results

6.2.1 Anthropometric Measures

The sum of 6 skinfolds and % body fat were less in ET than both UT and RT ($P < 0.05$, Table 6.1). RT tended to have a higher thigh cross-section area than UT (P =0.09, Table 6.1).

6.2.2 Incremental exercise performance

The ET had a higher peak workrate, and peak $\dot{V}O_2$, than both UT and RT. RT had a lower peak HR than UT ($P < 0.05$, Table 6.2).

Table 6.1 Anthropometric characteristics in untrained (UT) resistance trained (RT), and endurance trained (ET) subjects, Values are mean ± SD, n = 8 for each group.

	UT	**RT**	**ET**
Sum of 8 skinfolds (mm)	74.7 ± 24.4	58.3 ± 13.9	45.4 ± 16.3†‡
Body Fat (%)	14.5 ± 3.6	11.9 ± 2.3	9.4 ± 2.7†‡
Thigh muscle + bone C.S.A (cm^2)	207.5 ± 19.4	232.4 ± 16.2	219.9 ± 30.3

C.S.A, Cross-sectional area † different from UT; ‡ different from RT; all $P < 0.05$.

Table 6.2 Peak cardiorespiratory responses to incremental exercise in untrained (UT) resistance trained (RT), and endurance trained (ET) subjects; Values are mean ± SE, n = 8 for each group.

	UT	RT	ET
Workrate (W)	313 ± 11	334 ± 18	400 ± 18 †‡
HR ($b.min^{-1}$)	190 ± 3	185 ± 2†	188 ± 4
$\dot{V}_E$ ($l.min^{-1}$)	166.8 ± 7.0	154.1 ± 13.5	186.9 ± 7.6
$\dot{V}O_2$ ($l.min^{-1}$)	3.55 ± 0.14	3.53 ± 0.14	5.55 ± 0.28†‡
$\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$)	44.4 ± 1.8	43.8 ± 3.6	67.6 ± 1.5 †‡

† different from UT; ‡ different from RT $P < 0.05$.

6.2.3 Non-Invasive isokinetic muscle function

6.2.3.1 Torque-velocity response

RT had a higher peak torque than ET during dynamic isokinetic contractions (main effect, Fig 6.1), surprisingly however peak isometric torque did not differ significantly between the groups.

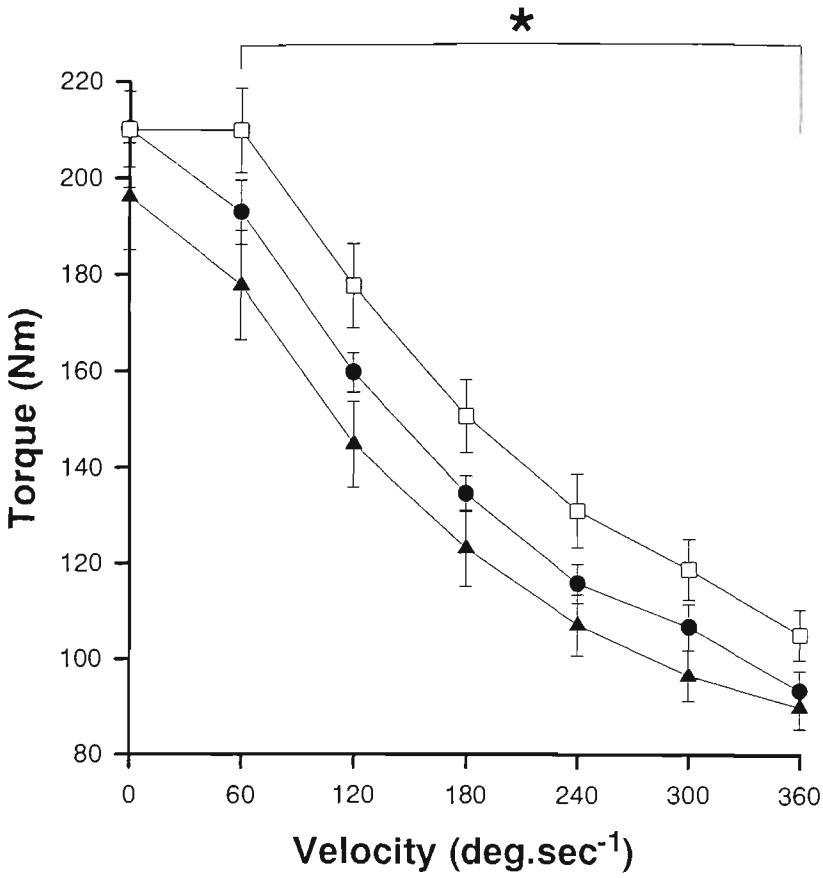

Figure 6.1 The torque-velocity response during isometric and isokinetic contractions on a Biodex dynamometer, in untrained (UT, ●), resistance trained (RT, □) and endurance trained (ET, ▲) subjects. * For dynamic contractions main effect for group RT > ET, UT ($P < 0.05$), n = 8, mean ± SE.

6.2.3.2 Muscle fatigue test

Performance variables measured during the non-invasive fatigue test completed on the Biodex isokinetic dynamometer are shown in Table 6.3. Peak work was higher in RT than UT and ET ($P < 0.05$), but no differences were evident between groups in peak torque. The fatigue index (FI) was lower in ET than in UT and RT, by ~27% and 25% respectively ($P < 0.05$).

Table 6.3 Non-invasive muscle fatigue test results in untrained (UT) resistance trained (RT), and endurance trained (ET) subjects, Values are mean ± SE, n = 8 for each group

	UT	RT	ET
Peak Torque (Nm)	165.7 ± 15.3	185.5 ± 18.5	168.3 ± 8.32
Peak Torque ($Nm.kg^{-1}$)	2.06 ± 0.11	2.27 ± 0.23	2.28 ± 0.14
Peak Work (J)	146.5 ± 4.7	153 ± 4.9†	118.9 ± 3.3 †‡
Peak Work ($J.kg^{-1}$)	1.84 ± 0.18	1.88 ± 0.06	1.61 ± 0.07
FI (%)	52.3 ± 3.5	50.1 ± 3.3	25.0 ± 5.6 †‡

† different from UT; ‡ different from RT $P < 0.05$.

6.2.3.3 Invasive isokinetic muscle fatigue test

Peak torque declined in all groups during the 50 contractions conducted on the Cybex dynamometer (Fig 6.2, $P < 0.05$). There were no significant differences in the FI between the Biodex and Cybex tests. The FI_{torque} for the UT, RT, and ET were 49.5 ± 2.8%, 55.6 ± 4.8%, 33.3 ± 5.3% respectively. The ET showed significantly lower FI than UT and RT ($P < 0.05$).

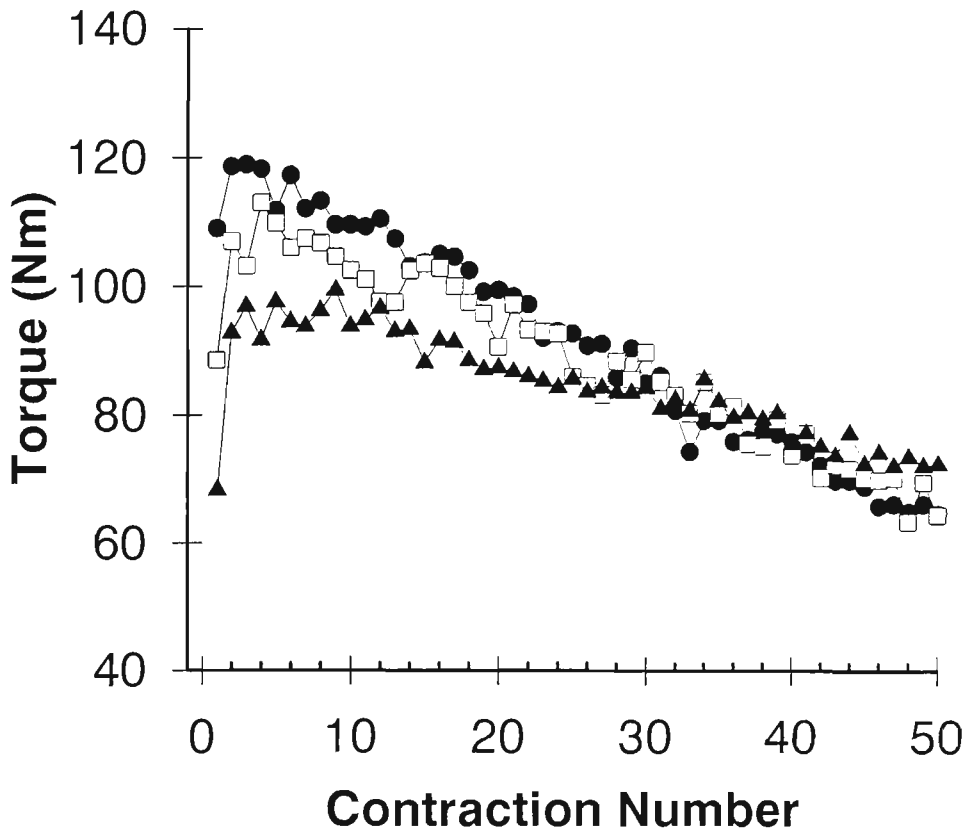

Figure 6.2 Knee extensor muscle peak torque for untrained (UT, ●), resistance trained (RT, □), and endurance trained (ET, ▲), subjects during 50 maximal contractions on a Cybex isokinetic dynamometer. Means shown but error bars omitted for clarity, n=8 for each group.

6.3 Muscle fibre type

ET had a higher proportion of type I fibres, compared to UT and RT ($P < 0.05$, Fig 6.3). RT and UT did not differ in fibre type proportion.

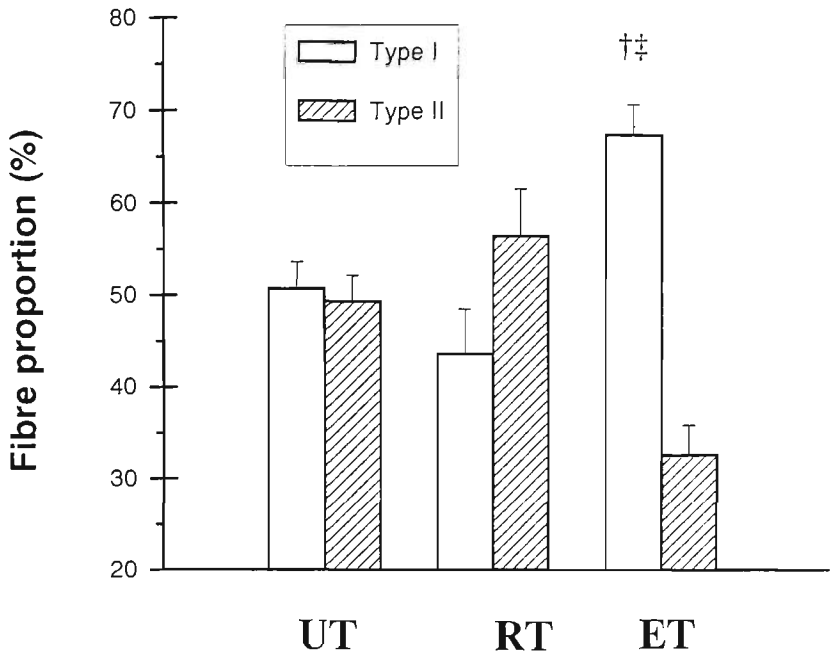

Figure 6.3 Muscle fibre proportions for untrained (UT) resistance trained (RT), and endurance trained (ET). †‡ ET > UT & RT ($P < 0.05$).

6.4 Maximal Na^+, K^+-ATPase activity

The resting skeletal muscle maximal *in-vitro* Na^+, K^+-ATPase activity did not differ between UT, RT and ET, when the results were expressed relative to muscle wet weight or per milligram of protein (P = 0.185, Table 6.4). However an independent t-test indicated 20.3% higher resting 3-*O*-MFPase activity in ET compared with UT (P<0.05). A significant main effect was found for fatigue effects on Na^+, K^+-ATPase, with the decline being 15.9%, ($P < 0.05$, Figure 6.4). No significant interaction was seen, indicating that the decline in Na^+, K^+-ATPase activity with fatigue was not different between the three groups. This fatigue-induced drop was also evident when the data was expressed relative to muscle protein content (-10.8%, $P < 0.05$).

Table 6.4 Vastus lateralis muscle maximal 3-*O*-MFPase activity (using 10mM KCl) at rest and at fatigue in untrained (UT) resistance trained (RT), and endurance trained (ET) subjects

	Na^+,K^+-ATPase activity (nmol min^{-1} g^{-1} wet wt.)		**Na^+,K^+-ATPase activity (pmol min^{-1} mg^{-1} protein)**	
Group	**Rest**	**Fatigue***	**Rest**	**Fatigue***
UT (n = 7)	207 ± 10	179 ± 9	1199 ± 54	1058 ± 49
RT (n = 8)	229 ± 19	205 ± 18	1295 ± 88	1180 ± 121
ET (n = 7)	249 ± 13	190 ± 12	1359 ± 44	1200 ± 84

Mean ± SEM * main effect fatigue < rest ($P < 0.05$) (Note the 3-*O*-MFPase activity data for UT subject #3, and ET #7 were incomplete and were excluded from the table and future results regarding 3-*O*-MFPase activity).

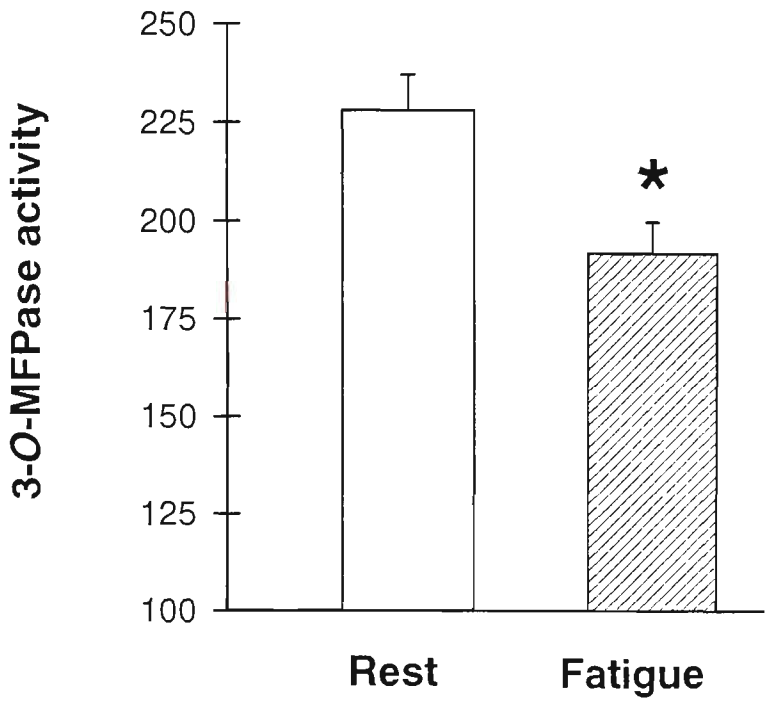

Figure 6.4 The maximal *in-vitro* 3-*O*-MFPase activity ($nmol.min^{-1}.g^{-1}$ wet wt.) in resting and fatigued skeletal muscle pooled for untrained (UT) resistance trained (RT), and endurance trained (ET). Data are mean ± SEM; n=22, * Main effect, Fatigue < Rest ($P < 0.05$).

6.4.1 Absolute and relative changes in Na^+, K^+-ATPase activity

To determine whether the depressive effects of exercise on Na^+, K^+-ATPase activity differed between groups, the absolute and percentage decline were compared. There were no differences between groups in the absolute or percentage change in Na^+, K^+-ATPase activity when the results were expressed relative to muscle wet weight or per milligram of protein (Table 6.5).

Table 6.5 Decline in 3-*O*-MFPase activity after MFT in untrained (UT) resistance trained (RT), and endurance trained (ET) subjects.

	Absolute decline in 3-*O*-MFPase activity		**Percent decline in 3-*O*-MFPase activity**	
	(nmol min^{-1} g^{-1} wet wt.)	**(pmol min^{-1} mg^{-1} protein)**	(%)	(%)
UT	27 ± 8	141 ± 26	13 ± 4	12 ± 2
RT	24 ± 13	115 ± 93	9 ± 6	8 ± 9
ET	60 ± 17	159 ± 88	22 ± 6	11 ± 6

Mean ± SEM, n = 7 for UT RT, ET.

6.5 Muscle Na^+, K^+-ATPase content

The Na^+, K^+-ATPase content for ET was 16.6% and 18.3% higher than UT and RT respectively ($P < 0.05$, Fig 6.5). There was a tendency for Na^+, K^+-ATPase content to be related to 3-*O*-MFPase activity relative to wet weight ($r = 0.34$, $P = 0.08$, Fig 6.6) and relative to protein ($r = 0.39$, $P = 0.09$).

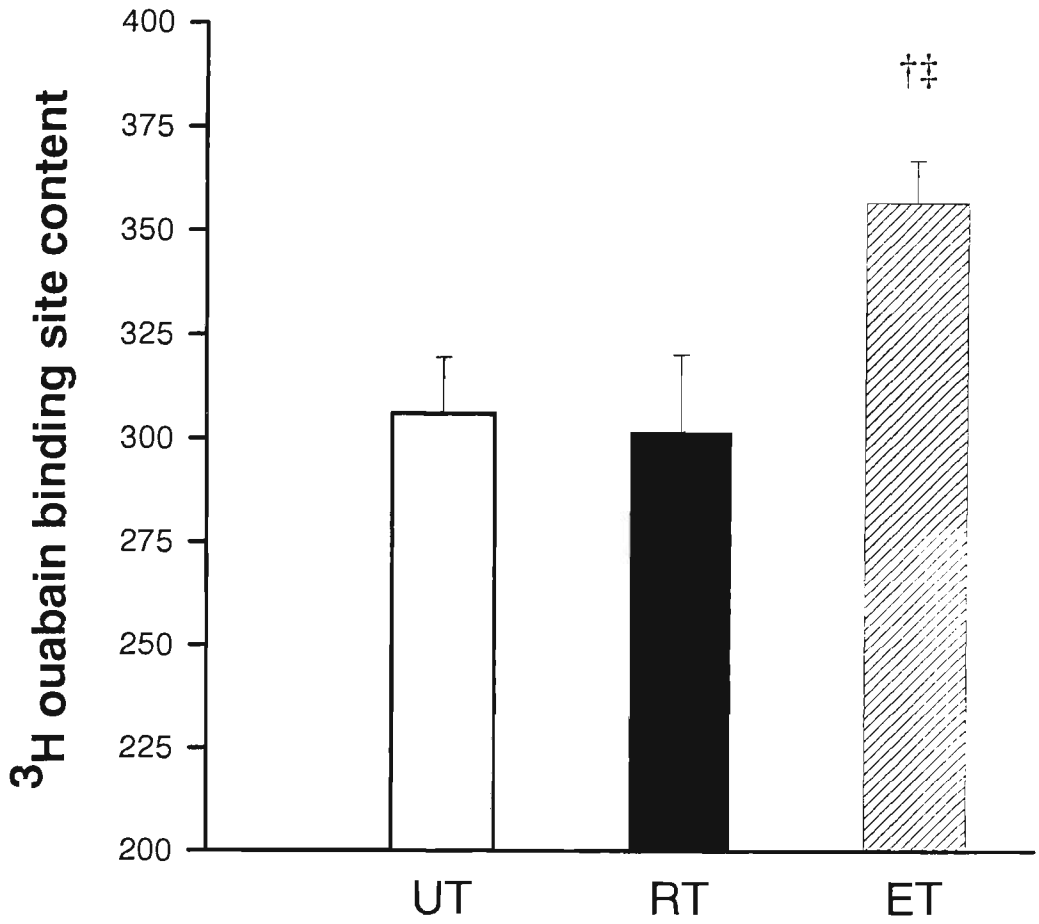

Figure 6.5 Na^+, K^+-ATPase content for untrained (UT) resistance trained (RT), and endurance trained (ET), n=8, Mean ± SEM, units $pmol.g^{-1}$ wet wt, * ET > UT and RT ($P < 0.05$).

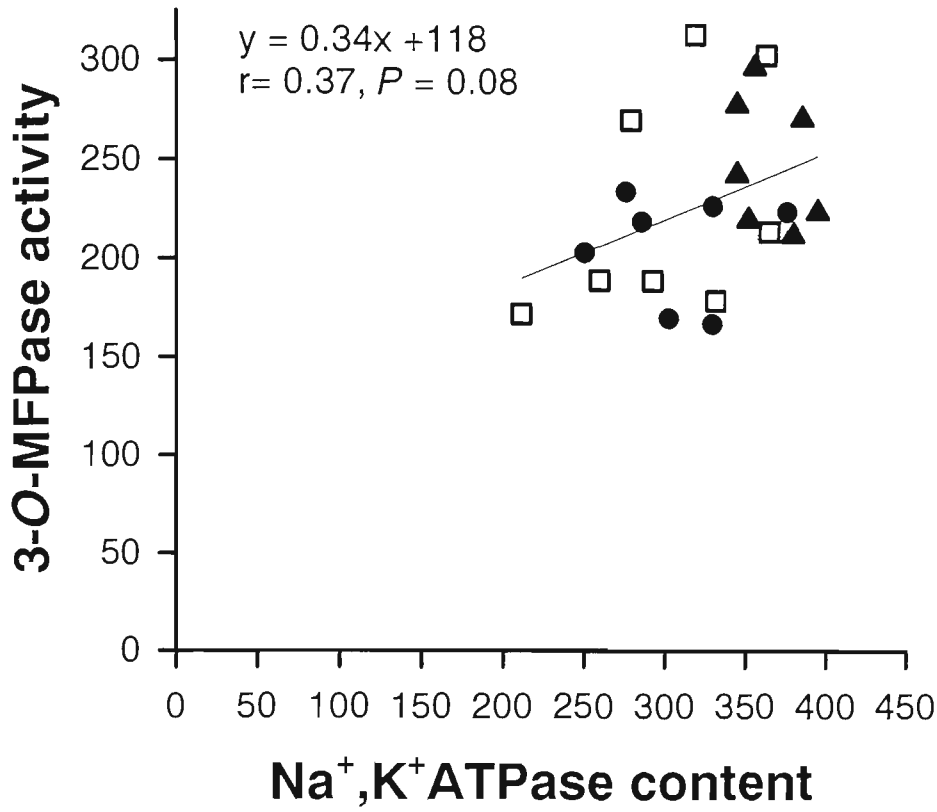

Figure 6.6 The relationship between Na^+, K^+-ATPase content (pmol.g^{-1} wet wt) and 3-*O*-MFPase activity (nmol min^{-1} g^{-1} wet wt) untrained (UT, ●), resistance trained (RT, □), and endurance trained (ET, ▲), n=22.

6.6 Muscle metabolites

Resting muscle

The only significant difference among the three groups in resting muscle metabolites was a higher PCr content in RT than in UT and ET ($P < 0.05$, Table 6.6).

Fatigued muscle

Muscle ATP content decreased with fatigue in UT and RT ($P < 0.05$), but this was not significant in ET. Muscle PCr and glycogen contents decreased, whilst lactate, Cr, IMP, and $[H^+]$ increased with fatigue in all groups ($P < 0.05$). IMP at fatigue was less in both

RT and ET compared to UT ($P < 0.05$). At fatigue, muscle lactate and Cr were less, whilst muscle PCr was greater, in ET compared to UT ($P < 0.05$).

Table 6.6 Skeletal muscle metabolites at rest and after fatigue in untrained (UT) resistance trained (RT), and endurance trained (ET) subjects.

	UT (n =8)		RT (n =8)		ET (n =6)	
	Rest	**Fatigue**	**Rest**	**Fatigue**	**Rest**	**Fatigue**
ATP	25.67 ± 0.85	19.51 ± 1.71*	26.28 ± 1.33	21.21 ± 1.29*	24.13 ± 1.37	20.80 ± 0.94
ADP	2.77 ± 0.18	2.90 ± 0.47	3.41 ± 0.24	3.52 ± 0.38	3.16 ± 0.25	3.58 ± 0.28
IMP*	0.08 ± 0.01	3.26 ± 0.84	0.09 ± 0.03	1.47 ± 0.62†	0.08 ± 0.01	2.56 ± 0.39 † ‡
PCr*	93.3 ± 2.3	35.8 ± 3.5	104.8 ± 2.9†	41.8 ± 3.4	82.3 ± 2.3‡	54.6 ± 3.3† ‡
Cr*	42.0 ± 2.6	99.5 ± 4.6	43.9 ± 3.3	108.3 ± 3.2	49.0 ± 4.3	76.7 ± 3.6† ‡
Lactate*	8.0 ± 0.8	91.6 ± 10.0	9.6 ± 1.0	84.8 ± 9.1	6.7 ± 0.9	66.1 ± 7.2† ‡
$[H^+]$*	68.4 ± 2.7	152.6 ± 13.3	73.2 ± 3.5	185.2 ± 9.3	65.7 ± 3.2	133.3 ± 9.7
Glycogen*	469 ± 34	356 ± 30	498 ± 34	376 ± 21	510 ± 32	349 ± 29

Values are expressed as $mmol.kg^{-1}$ dry weight of muscle, except $[H^+]$ ($nmol.L^{-1}$) and glycogen (mmol glucosyl $units.kg^{-1}$ dry muscle), Mean ± SEM; * Main effect rest different from fatigue ($P < 0.05$); † different from UT corresponding sample; ‡ different from RT corresponding sample, $P < 0.05$.

6.7 Relationships between muscle 3-*O*-MFPase activity, Na^+, K^+-ATPase content, muscle function and fibre type

When data from the three groups were combined there was a significant relationship between 3-*O*-MFPase activity and percentage of type I fibres ($r = 0.53$, $P < 0.05$, Fig 6.7). However, there was no relationship between Na^+, K^+-ATPase content and muscle fibre type. A weak significant inverse relationship was found between Na^+,K^+-ATPase content

and fatigue index during the MFT (r= -0.42, P < 0.05, Fig 6.8). There were no other relationships between MFT peak torque, fatigue index, or work done, and 3-*O*-MFPase activity or Na^+, K^+-ATPase content for the three groups.

6.7.1 Relationships between 3-*O*-MFPase activity and muscle metabolites at rest and fatigue

There were no significant relationships between resting muscle 3-*O*-MFPase activity and muscle metabolites at rest or fatigue. When data for all three groups was pooled this also failed to show any relationships. The absolute changes in 3-*O*-MFPase activity and absolute changes in muscle metabolites during the MFT were not significantly related. However, the percentage drop in 3-*O*-MFPase activity was correlated to the percentage drop in muscle glycogen ($r = 0.53$, $P < 0.05$). There were no other relationships between 3-*O*-MFPase activity or Na^+, K^+-ATPase content and the percentage change in muscle metabolites from rest to fatigue.

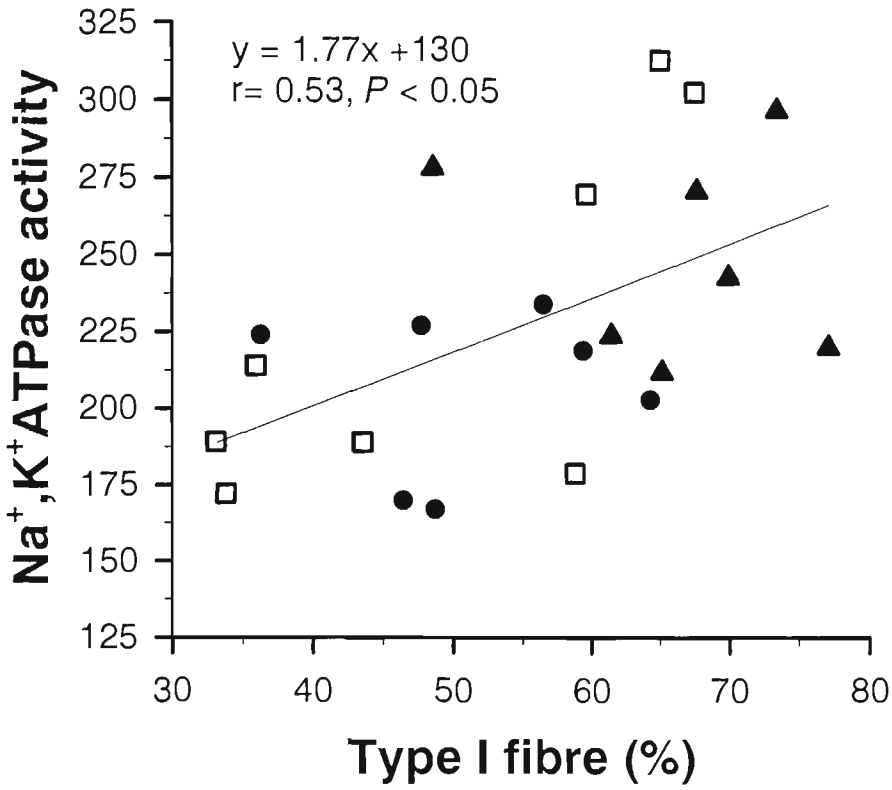

Figure 6.7 The relationships between 3-*O*-MFPase activity (nmol min^{-1} g^{-1} wet wt) and type 1 fibre percent for untrained (UT, ●), resistance trained (RT, □), and endurance trained (ET, ▲) subjects (n=22).

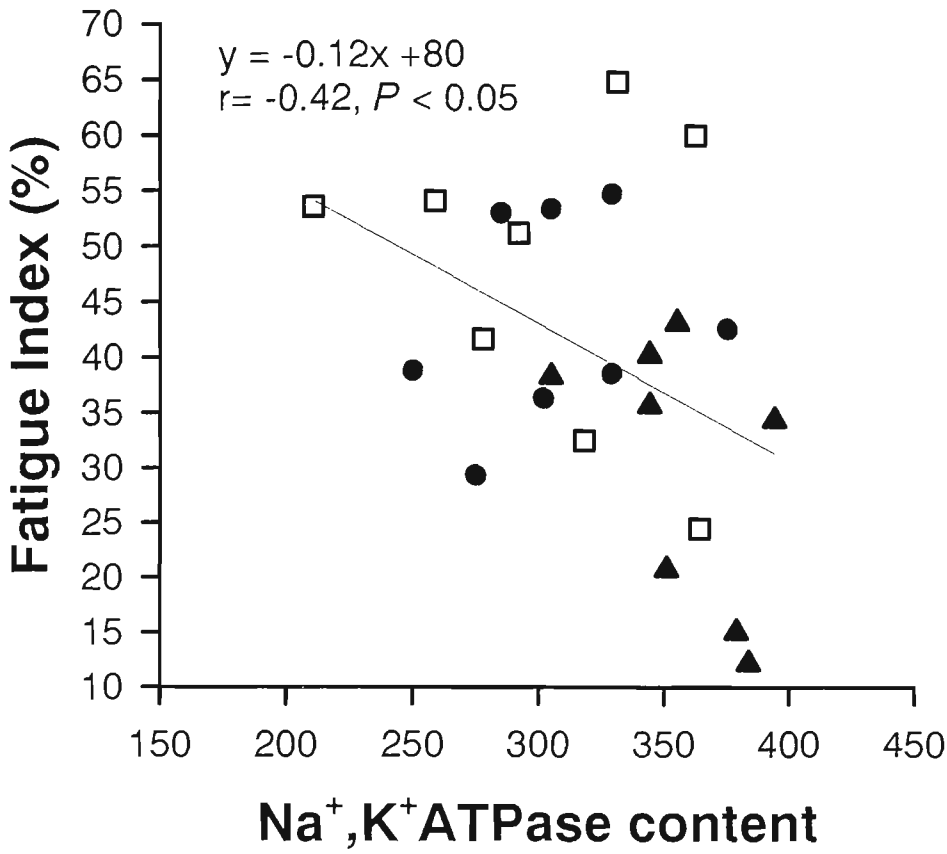

Figure 6.8 The relationship between Na^+, K^+-ATPase content ($pmol.g^{-1}$ wet wt) and Fatigue Index during the muscle fatigue test for untrained (UT, ●), resistance trained (RT, □), and endurance trained (ET, ▲),n=24.

6.8 Plasma volume and electrolyte changes during muscle fatigue test

6.8.1 Plasma volume, plasma [lactate] and $[H^+]$

Plasma volume decreased significantly below resting levels at the end of the MFT and continued to fall until 2 min of recovery ($P < 0.05$). It had returned to within resting levels by 10 min recovery. There were no significant differences between groups.

A small rise in plasma $[H^+]$ occurred at the end of MFT, peaked at 5 min post and had returned to within resting levels by 20 min post exercise. (Figure 6.9A). ET recovered faster than UT and RT, with $[H^+]$ lower than UT and RT at 5 min post and had returned to

resting levels by 10 min post. Plasma lactate rose three-fold during the 50 contractions, peaked 1-2 min into recovery and slowly declined until plasma lactate did not differ from resting levels by 20 min post for UT and RT (Figure 6.9B). Peak [lactate] was less and ET recovered faster than UT and RT where plasma lactate was lower than UT and RT at 5 min post and had returned to resting levels by 10 min post.

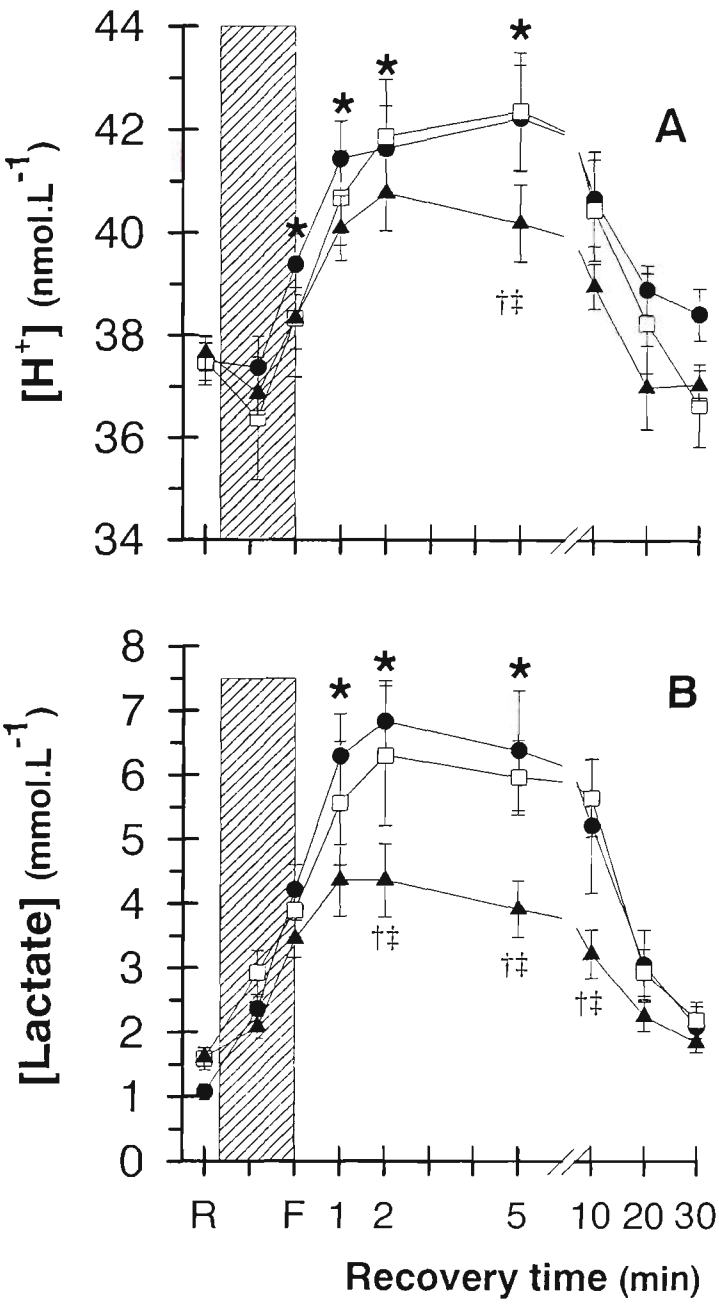

Figure 6.9 (**A**) Plasma $[H^+]$ and (**B**) lactate at rest (R), mid exercise and at the point of fatigue (F) in the muscle fatigue test and 30 min of recovery for untrained (UT, ●), resistance trained (RT, □) and endurance trained (ET, ▲) subjects. Data: n = 8 for UT and ET, n =7 for RT, Mean ± SEM. * main effect all groups different from rest ($P < 0.05$). †‡ ET < RT and UT ($P < 0.05$).

6.8.1.2 Plasma K^+ response during MFT

Arterialised-venous plasma $[K^+]$ increased from rest and peaked at the end of the MFT (Figure 6.10), but did not differ between the UT, RT and ET groups. The peak $[K^+]$, $\Delta[K^+]$ and $\Delta[K^+].work^{-1}$ were not different between the three groups (Table 6.7).

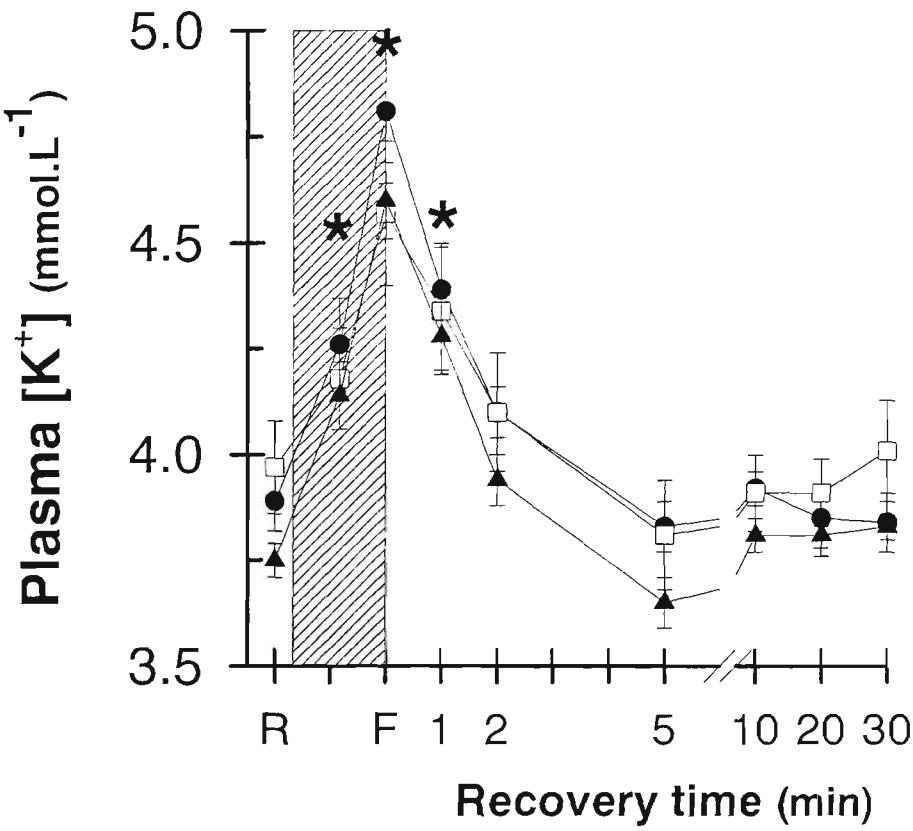

Figure 6.10 Plasma K^+ concentration at rest (R), mid and at the point of fatigue (F) in the MFT and 30 min of recovery for untrained (UT, ●), resistance trained (RT, □) and endurance trained (ET, ▲) subjects. Data: n = 8 for UT and ET, n =7 for RT, Mean ± SEM. * main effect all groups higher from rest ($P < 0.05$).

Table 6.7 Plasma $[K^+]$ variables during the MFT for untrained (UT) resistance trained (RT), and endurance trained (ET) subjects. n = 8 for UT and ET, n =7 for RT.

	UT	RT	ET
Peak $[K^+]$ ($mmol.L^{-1}$)	4.81 ± 0.17	4.57 ± 0.17	4.60 ± 0.09
$\Delta[K^+]$ ($mmol.L^{-1}$)	0.92 ± 0.13	0.60 ± 0.12	0.85± 0.09
$\Delta[K^+].work^{-1}$ ($nmol.l^{-1}.J^{-1}$)	85.7 ± 13.0	59.7 ± 11.6	77.6 ± 8.5

6.8.2 Relationship between 3-*O*-MFPase activity, Na^+,K^+-ATPase content, and K^+ regulation during MFT

There were no significant relationships between 3-*O*-MFPase activity or Na^+, K^+-ATPase content and $\Delta[K^+]$ or $\Delta[K^+].work^{-1}$ for the MFT for the three groups.

6.8.3 Plasma volume and $[K^+]$ response during incremental exercise test

There was no difference in the plasma volume response from rest to $\dot{V}O_2$ peak during the incremental exercise test between the three groups (% ΔPV: UT -13.4 ± 1.5, RT -12.1 ± 1.4, ET -12.3 ± 2.6). The arterialised-venous plasma $[K^+]$ increased from rest to peak exercise but the response was not different between the 3 groups (Figure 6.11). Whilst peak $[K^+]$ and $\Delta[K^+]$ were not different between the groups, the $\Delta[K^+].work^{-1}$ ratio was 36% lower in ET (11.8 ± 0.4 $nmol.J^{-1}$) compared to UT (18.5 ± 2.3 $nmol.J^{-1}$, $P < 0.05$), and also tended to be lower than in RT (16.2 ± 2.2 $nmol.J^{-1}$, $P = 0.09$).

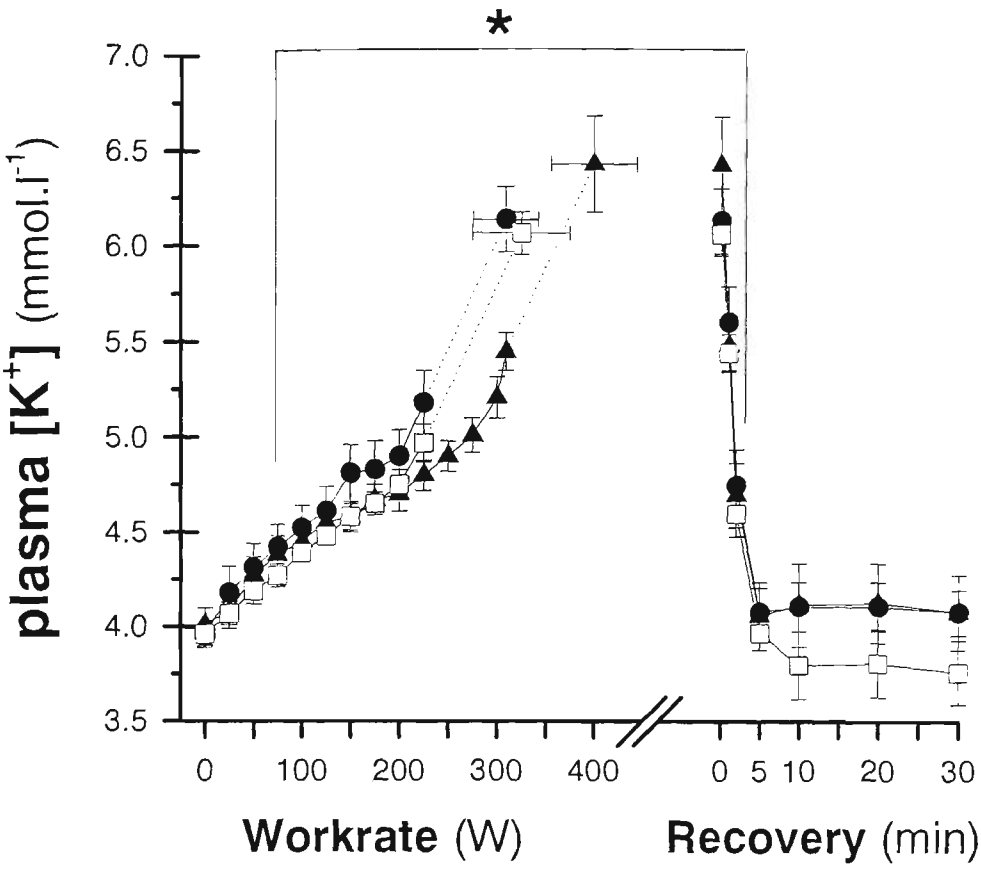

Figure 6.11 Plasma $[K^+]$ at rest, during the incremental test and during 30 min of recovery for untrained (UT, ●), n=8, resistance trained (RT, □), n=7, and endurance trained (ET, ▲), n=8 subjects. Horizontal error bars for peak values indicate mean ± SEM workrate. The zero recovery time point is the peak exercise value replotted. * significant main effect for all groups different from rest ($P < 0.05$).

6.8.4 Relationships between 3-*O*-MFPase activity, Na^+, K^+-ATPase content, $\dot{V}O_2$ peak, and muscle fibre type

Both muscle 3-*O*-MFPase activity expressed relative to wet weight and Na^+, K^+-ATPase content were significantly correlated with $\dot{V}O_2$ peak (r=0.46, r=0.65, respectively, $P < 0.05$, Fig 6.12A,B). There was also a relationship between $\dot{V}O_2$ peak relative to body mass and the percentage of type I fibres (r = 0.62, $P < 0.05$, Fig 6.12C).

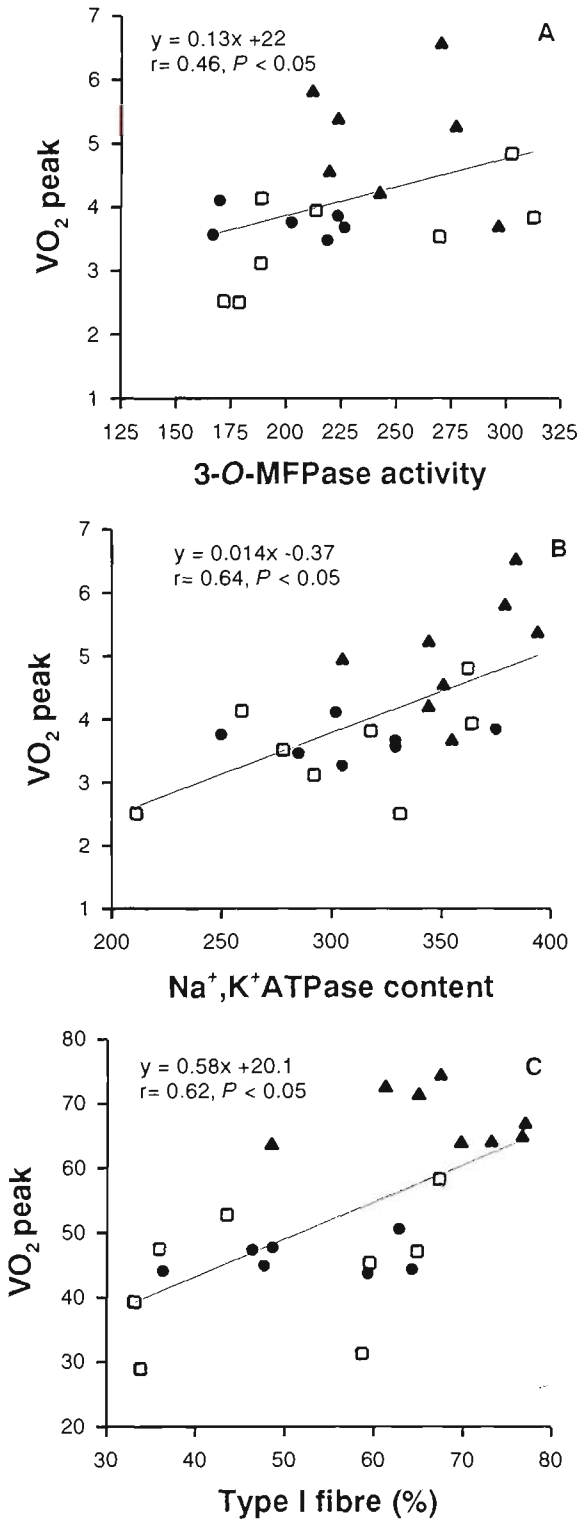

Figure 6.12 Relationship between $\dot{V}O_2$ peak ($l.min^{-1}$) and (**A**) 3-*O*-MFPase activity (nmol min^{-1} g^{-1} wet wt) (**B**) (Na^+, K^+-ATPase content (pmol.g^{-1}wet weight) and (**C**) % type I fibres for pooled untrained (UT, ●), resistance trained (RT, □), and endurance trained (ET, ▲), n=23, (n = 22 for A, $\dot{V}O_2$ peak for C reported in $ml.kg^{-1}.min^{-1}$).

6.8.5 Relationship between muscle 3-*O*-MFPase activity, Na^+,K^+-ATPase content, and plasma $[K^+]$ during the incremental exercise test

The $\Delta[K^+].work^{-1}$ ratio was inversely related to 3-*O*-MFPase activity both when expressed relative to muscle wet weight (r = -0.53, $P < 0.05$, Fig 6.13A) and muscle protein (r = -0.57, $P < 0.05$) during the incremental exercise test. There was also a significant inverse relationship between Na^+, K^+-ATPase content and $\Delta[K^+].work^{-1}$ during the incremental test (r = -0.49, $P < 0.05$, Fig 6.13B). There was also a tendency for 3-*O*-MFPase activity to correlate with peak work during the incremental test (r = 0.39, P = 0.07) whilst Na^+, K^+-ATPase content was related to peak work (r = 0.53, $P < 0.05$).

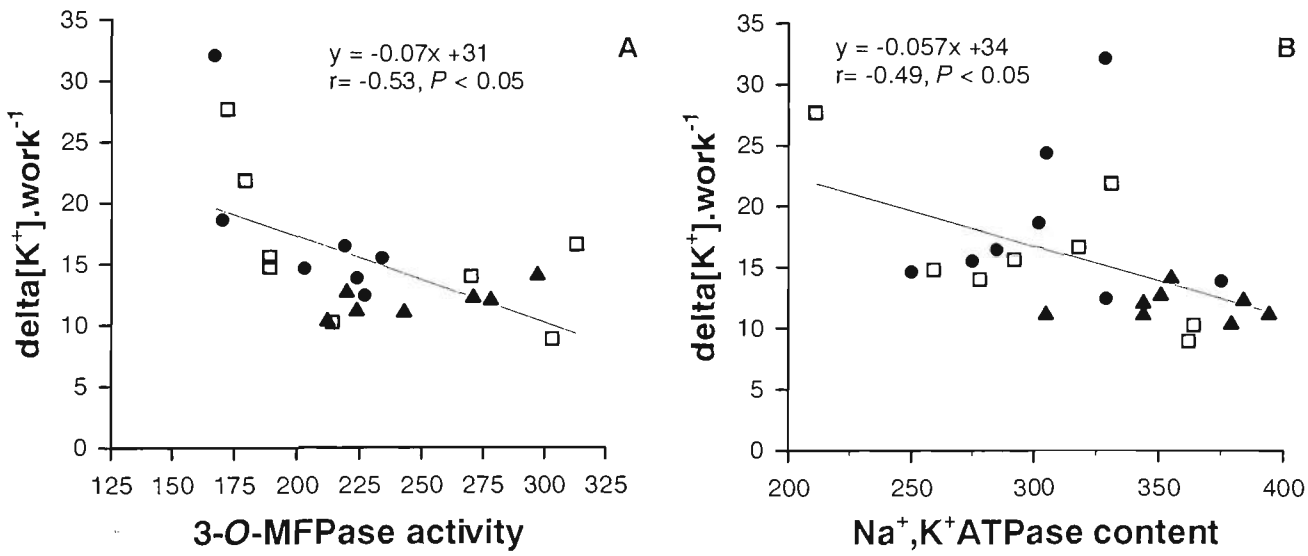

Figure 6.13 The relationship between $\Delta[K^+].work^{-1}$ ($nmol.l^{-1}.J^{-1}$) and (**A**) 3-*O*-MFPase activity (nmol.min-1.g^{-1} wet wt, n=22) and (**B**) Na^+, K^+-ATPase content ($pmol.g^{-1}$ wet wt, n=24) for the incremental test untrained (UT, ●), resistance trained (RT, □), and endurance trained (ET, ▲).

6.9 Discussion

The hypothesis that training reduces the decline in maximal *in vitro* Na^+, K^+-ATPase activity with fatigue was not supported by the findings of this study. This confirms the finding of an acute depression in Na^+, K^+-ATPase activity with fatigue shown in the previous chapter (5) with a larger subject population. This suggests that the observed depression in Na^+, K^+-ATPase activity with fatigue is an obligatory response with fatiguing muscular contractions, irrespective of training status. This study was also the first to show a relationship between Na^+, K^+-ATPase activity and type I fibres in human skeletal muscle.

6.9.1 Training does not prevent reduced Na^+, K^+-ATPase activity with fatigue

The important finding from this study was the similar decrease in Na^+, K^+-ATPase activity whether expressed in relative or absolute terms for UT, RT, and ET. This indicates that training status has no clear effects on the depression in Na^+, K^+-ATPase with fatiguing contractions. Thus the implied structural alterations to the Na^+, K^+-ATPase enzyme observed in UT, occurred in the trained subjects. Unfortunately the extent to which intracellular Ca^{2+}, temperature, oxygen free radicals or metabolic perturbations effect the structural integrity of the Na^+, K^+-ATPase cannot be qualified here. However the effects of training on some of these processes have been documented, therefore allowing speculation as to their importance.

6.9.1.1 Metabolic changes

The percentage decline in Na^+, K^+-ATPase activity with fatigue across all groups was associated with the percentage change in muscle glycogen levels. Further, there was a

weak relationship between Na^+, K^+-ATPase activity and the fall in PCr levels and rise in lactate levels with fatigue. As stated in Chapter 5, these metabolic changes may merely reflect an association between parallel changes with fatigue. These metabolic disturbances would only effect the *in vitro* Na^+, K^+-ATPase measure if they altered the enzymatic activity (eg alter structural integrity) of the enzyme. Despite this, if they are important in the etiology of fatigue-induced reductions in Na^+, K^+-ATPase activity, they are likely to be even greater in the *in vivo* situation.

The link between glycolysis and Na^+, K^+-ATPase activity was shown in rat skeletal muscle (James et al. 1996; 1999). James et al. (1996; 1999) indicated that the stimulation of Na^+, K^+-ATPase was linked to stimulation of glycolysis and the increase in lactate production under fully aerobic conditions and this was more marked in the EDL that in the oxidative soleus muscle. These authors also implied that a significant fraction of the ATP to fuel the Na^+, K^+-ATPase must be supplied by glycolysis, and a high degree of metabolic compartmentalisation occurs (James et al. 1996). A local fall in ATP concentration may partially inhibit Na^+, K^+-ATPase activity, as shown in cultured epithelial cells in the rabbit (Kuwahara et al. 1998). Kuwahara and colleagues (1998) showed that an episode of ATP depletion initiated by metabolic inhibitors such as rotenone, caused a partial inhibition of Na^+, K^+-ATPase activity (^{86}Rb uptake). The Na^+, K^+-ATPase is reported to account for 20-30% of cellular ATP hydrolysis (Siems et al. 1984; Buck & Hochachka 1993). Therefore any local fall in ATP concentration is likely to affect the Na^+, K^+-ATPase but the exact mechanism is not clear. Some investigators have suggested that lowered cellular ATP concentration can alter the association of Na^+, K^+-ATPase with the cytoskeleton (Molitoris et al. 1992) and cause internalisation of Na^+, K^+-ATPase molecules (Mandel et al. 1994). Endurance training would allow improved regulation ATP production and utilisation, as evidenced by the ET group in this study

showing a non significant drop in cellular ATP compared to the other two groups. However the reduction in Na^+, K^+-ATPase activity with fatigue was not attenuated in any of the groups indicating at least that training effects on global metabolic measures (ie glycogen, ATP etc) did not significantly effect Na^+, K^+-ATPase activity. This does not rule out local metabolic effects potentially compromising Na^+, K^+-ATPase activity and/or exacerbating the effects of the *in vitro* decline in Na^+, K^+-ATPase activity in the *in vivo* situation.

6.9.1.2 Reactive Oxygen species

Aerobic production of energy results in a proportional rise in reactive oxygen species, while endurance training results in higher $\dot{V}O_2$ values than untrained individuals, thus a higher production of ROS is likely. However this is balanced by an increase in antioxidant enzyme activity (Powers & Lennon 1999; Vincent et al. 2000). Increases in both enzymatic and non-enzymatic cellular defence mechanisms occur, but the increase is restricted to highly oxidative fibres (Ji et al. 1988, Powers et al. 1994). The antioxidant enzymes superoxide dismutase, GPX (GSH peroxidase) but not catalase activities increase in trained skeletal muscle (Ji et al. 1988; Leewenburgh et al. 1994; 1997; Powers et al. 1994). Studies on non-enzymatic antioxidants and training are inconsistent but there is evidence for training to increase GSH and ubiquinone levels especially in rat and human oxidative fibres (Beyer et al. 1984; Karlsson et al. 1996; Leewenburgh et al. 1994; 1997; Powers & Lennon 1999). The benefits of ET in protecting against the deleterious ROS effects on the Na^+, K^+-ATPase during the muscle fatigue test remains unclear. Further, no studies have investigated the effect of any of these factors on the Na^+, K^+-ATPase during exercise.

6.9.1.3 Ca^{2+} inactivation of Na^+, K^+-ATPase activity

As outlined in Chapter 5, a possible mechanism for the depressed Na^+, K^+-ATPase activity with fatigue is a rise in intracellular $[Ca^{2+}]$ (Breier et al. 1998; Sulová et al. 1998). Calcium ions are involved in the regulation of many processes in excitable cells (Breier et al. 1998; Racay et al. 1996; Sulová et al. 1998). In millimolar concentrations Ca^{2+} inhibits both the Na^+, K^+-ATPase ATPase hydrolytic activity (Huang & Askari 1982; Yingst et al. 1992) and transport activity of the enzyme (Stankovicová et al. 1995). The effect of training on intracellular $[Ca^{2+}]$ is unknown and it is not possible from this study to elucidate the role Ca^{2+} plays in the fatigue-induced depression seen in the untrained and the chronically trained groups. However a higher proportion of type II fibres has been associated with a greater density of Na^+ channels (Ruff 1996). This would invoke greater entry of Ca^{2+} during contraction, giving rise to a greater cytosolic $[Ca^{2+}]$. This raised cytosolic $[Ca^{2+}]$ is likely to have invoke more deleterious effects on the Na^+, K^+-ATPase. However the ET actually tended to show the greatest decline in Na^+, K^+-ATPase activity with fatigue, and paradoxically had the lowest percentage of type II fibres.

6.9.1.4 Fibre type

The increased proportion of oxidative fibres with ET was postulated to protect against the deleterious effects of fatigue on Na^+, K^+-ATPase. In this study, despite a relationship between type I fibre proportion and Na^+, K^+-ATPase activity, this did not invoke protection against the detrimental effects of fatiguing exercise on Na^+, K^+-ATPase activity. Green & Chin (1993) have shown a relationship between oxidative potential and Na^+, K^+-ATPase content in rat skeletal muscle, however others have reported no clear indication of a Na^+, K^+-ATPase fibre type dependence in human skeletal muscle (Benders

et al. 1992; Dørup et al. 1988; Madsen et al. 1994; Nørgaard 1986). Similarly, no such finding was found in this study. Opposed to these finding however, a new finding in this study is a relationship between oxidative fibres and Na^+, K^+-ATPase activity.

6.9.2 Na^+, K^+-ATPase content and training status

Despite the lack of a significant effect of training status on Na^+, K^+-ATPase activity, the endurance trained group showed a significantly higher Na^+, K^+-ATPase content (17%). This ~17% higher Na^+, K^+-ATPase content is consistent with other endurance training studies (Green et al. 1993; Evertsen et al. 1997; Madsen et al. 1994). No increase in Na^+, K^+-ATPase content was found in the RT group, which is contrary to the 16% increase with RT (Green et al. 1999a) and 45% higher Na^+, K^+-ATPase content in chronic resistance trained older men (Klitgaard & Clausen 1989) respectively. The UT subjects average value for ^3H ouabain binding site content was 306±18 pmol.g^{-1} wet wt which is lower than other published values for untrained (or moderately active subjects) such as 333 and 339 pmol.g^{-1} wet wt for McKenna et al. 1993 and Green et al.1993 respectively. Therefore it does not appear that our untrained subjects were a degree of absence from a sedentary state. The ET group were clearly more trained than UT with a 56% higher $\dot{V}O_2$ peak, however an unexpected limitation in this study was that the RT group did not differ greatly from UT in muscle mass or peak muscle torque, and the only clear difference being a greater dynamic torque generated. Unfortunately I was unable to recruit elite weight lifters as they were unwilling to undergo muscle biopsies. A functional criterion was used to differentiate RT from recreational gym users, but this may not have been stringent enough. Thus, the lack of an effect of RT on Na^+, K^+-ATPase activity and content could be that this group was insufficiently trained in comparison to previous studies (Green et al. 1999a; Klitgaard & Clausen 1989). To investigate this question

further, a more highly trained group is required and a longitudinal design (eg Green et al. 1999a) may be more beneficial. Further, no measurements were made to confirm possible muscle fibre hypertrophy. The muscle fibre hypertrophy resulting from RT will result in less sarcolemmal surface area for a given mass of tissue (Harrison et al. 1994). Thus any increase in Na^+, K^+-ATPase enzyme number with resistance training may be diluted by the increase in fibre cross sectional area relative to sarcolemmal area.

6.9.2.1 Na^+, K^+-ATPase content vs activity

A surprising finding was the lack of a strong relationship ($r = 0.37$, $P < 0.05$) between Na^+, K^+-ATPase content and activity. The variability associated with each measure and the relatively small spread of data, may each have contributed to this weak relationship. When another group (age-matched controls for transplant patients, Chapter 7) was added the relationship was much stronger ($r = 0.61$, $P < 0.05$). The direct association between Na^+, K^+-ATPase activity and Na^+, K^+-ATPase content has previously been established in rat skeletal muscle (Nørgaard et al. 1984b). In the Nørgaard et al. study (1984b) a molar activity or turnover number was calculated but in order to do this activity and content were determined from the same purified homogenate. The previously reported molecular activity values of 620 min^{-1} was based on a partially purified muscle extracts (Nørgaard et al. 1984b). Utilising this turnover value for our Na^+, K^+-ATPase activity data gives values in the range of 330-400 $pmol.g^{-1}$ wet wt. which is comparable to our Na^+, K^+-ATPase content determination of 300-360 $pmol.g^{-1}$ wet wt. The discrepancy is likely to lie in the fact that the 3-*O*-MFPase activity assay used here was modified from previously published techniques (Benders et al. 1992; Nørgaard et al. 1984b). Therefore a new molecular activity should be calculated for the changed conditions of the assay measuring 3-*O*-MFPase activity and $[^3H]$-ouabain binding site content from the same homogenate, if

one wants to convert between activity and content. As this precise measurement was not performed, it is inappropriate to convert between Na^+, K^+-ATPase activity and content based on an assumed molecular activity. Further this calculation has not been performed as this study had independent measures of content and activity and the need to convert between the two measures is not required. It has previously been shown that it is not always appropriate to merely convert between the two distinct measures (Druml et al. 1988; Pickar et al. 1994).

6.9.3 Relationship between muscle Na^+, K^+-ATPase and performance

ET had a higher Na^+, K^+-ATPase content and also showed a smaller percentage drop in peak torque during the fatiguing exercise bout. There was also an inverse relationship between Na^+, K^+-ATPase content and the fatigue index. This finding differs from Klitgaard & Clausen (1989) who showed no correlation with time to fatigue when performing submaximal knee extensor contractions at 60% of max isometric force. Madsen et al. (1994) also failed to show a relationship between the increase in Na^+, K^+-ATPase content with intensified ET and the increase in endurance time after intensified training. The lack of association between the decline in Na^+, K^+-ATPase activity and muscle fatigue index may be due to the fact that Na^+, K^+-ATPase activity loss with fatigue may be greater in the *in vivo* situation. However other factors are clearly involved in the fatigue process including depressed SR Ca^{2+} release and re-uptake, and the deleterious metabolic and contractile effects of increased intracellular $[H^+]$ and P*i* (Fitts 1994).

The relationship between Na^+, K^+-ATPase content with $\dot{V}O_2$ peak supports the findings of previous human training studies (Evertsen et al. 1997; Green et al. 1999a; Madsen et al. 1994). In addition, this study also showed that maximal Na^+, K^+-ATPase activity was

related to $\dot{V}O_2$ peak. However, as with most of the above studies the relationship between this performance parameter and Na^+, K^+-ATPase did not account for a large proportion of the variance, indicating that other factors are clearly involved in determining maximal O_2 uptake. There were no other associations between Na^+, K^+-ATPase content or activity and muscle function.

6.9.4 Na^+, K^+-ATPase function and plasma $[K^+]$

Previous studies have investigated the relationship between the decreased plasma $[K^+]$ and increased Na^+, K^+-ATPase content after training (McKenna et al. 1993; Madsen et al. 1994). The ET subjects displayed a reduced $\Delta[K^+].work^{-1}$ ratio during the incremental test compared to the UT subjects. These findings support the reduced $\Delta[K^+].work^{-1}$ seen after sprint training (Harmer et al. 2000; McKenna et al. 1993) and reduced hyperkalemia after endurance training (Green et al. 1993; Kjeldsen et al. 1990a, Tibes et al. 1974). This decrease in $\Delta[K^+].work^{-1}$ during the incremental test was associated with the higher Na^+, K^+-ATPase content seen in this group. It is possible that the reduced $\Delta[K^+].work^{-1}$ was due to reduced release of K^+ from exercising muscle but this is less likely that the explanation that increased Na^+, K^+-ATPase content or activity in exercising and non exercising muscle would clear more extracellular $[K^+]$ thus reduce the $\Delta[K^+].work^{-1}$ (Evertsen et al. 1997).

The reduced $\Delta[K^+].work^{-1}$ seen in the incremental test was not evident during MFT. Possible reasons for this discrepancy may lie in the differences in muscle mass recruited relative to work done for the two tests. As mentioned in the previous chapter, the rise in circulating $[K^+]$ during one leg maximal exercise is lower, but the relationship between Na^+, K^+-ATPase and circulating $[K^+]$ may be more evident when looking at the $[K^+]$ response during the incremental exercise test. This test utilising a larger muscle mass,

induced a greater rise in [K^+] and possibly an increased activation of the Na^+, K^+-ATPase. Further the ET group are more likely to display performance adaptions in response to training when challenged with more aerobic type exercise.

Similar to the comments in chapter 5, interpretation of relationships between plasma [K^+] and Na^+, K^+-ATPase activity or content should be interpreted with caution. There are limitations in the use of arterialised-venous blood samples, as well as the choice of exercise test. Further, the correlations were generally weak, indicating that other factors are clearly involved in regulating plasma [K^+] during exercise. These include, muscle blood flow, K^+ clearance by active and inactive tissues.

6.9.5 Conclusion

In summary, training did not alter resting Na^+, K^+-ATPase activity. The RT did not differ from UT in relation to Na^+, K^+-ATPase content or activity or performance parameters, whilst both forms of training did not attenuate or prevent the factors compromising Na^+, K^+-ATPase activity during fatiguing exercise. However the endurance trained had a higher Na^+, K^+-ATPase content and there were significant relationships between Na^+, K^+-ATPase activity and type I fibre percentage, muscle fatigue index and $\dot{V}O_2$ peak. The inverse relationship between $\Delta[K^+].work^{-1}$ during the incremental test and Na^+, K^+-ATPase activity or content confirms the important role the Na^+, K^+-ATPase in the plasma [K^+] response.

Chapter 7 Thoracic organ transplantation and Na^+,K^+-ATPase function

7.0 Introduction

Recipients of thoracic transplants eg heart, heart-lung, and single or double lung transplants, suffer from severe exercise limitation both before and after transplantation. For a considerable period of time before transplantation, these patients are usually chronically inactive and thus serve as an interesting model for muscular disuse. Cardiopulmonary testing of transplant patients indicates a low peak work rate, early lactate threshold and peak O_2 consumption which are around one-half of predicted values for the healthy population (Ambrosino et al. 1996; Banner et al. 1989; Miyoshi et al. 1990; Theodore et al. 1987). Lung transplantation appears successful in removing the ventilatory limitation to exercise (Williams et al. 1992), so other reasons are given for this exercise limitation, including poor motivation, low [haemoglobin], impact of immunosuppressive medication and peripheral muscle defects (Gibbons et al. 1991; Otulana et al.1992; Ross et al. 1993; Theodore et al. 1987).

One of the peripheral muscle defects postulated to limit exercise capacity in transplant patients is altered Na^+, K^+-ATPase function (Hall et al. 1994). In Hall and colleagues' study (1994), heart transplant patients displayed impaired plasma K^+ regulation with a higher $\Delta[K^+].work^{-1}$ ratio during incremental exercise compared to matched controls. Nørgaard et al. (1990) showed that patients with congestive heart failure had 25% lower

skeletal muscle Na^+, K^+-ATPase content and Barlow et al. (1994) also showed that such patients had higher arterial $[K^+]$ at similar low workloads to controls. Muscle disuse and chronic inactivity lead to altered muscle morphology (see table 2.4), and lower Na^+, K^+-ATPase content (see table 2.5).

In Chapter 6 it was shown that chronic endurance training increased Na^+, K^+-ATPase content and that this was correlated with $\dot{V}O_2$ peak. The endurance trained individuals also had a higher proportion of type I fibres but this did not reflect in increased maximal *in vitro* Na^+, K^+-ATPase activity or $[K^+]$ regulation. In humans, 6 weeks of immobilisation increased the proportion of soleus type II fibres from 2.4% to 10.7% (Haggmark & Eriksson 1979). It was of interest to note if a transplant patient group characterised by chronic levels of inactivity, showed altered exercise performance, muscle fibre composition, Na^+, K^+-ATPase activity and $[K^+]$ regulation. Thus transplant patients serve as an interesting model where the ventilatory limitation to exercise has been removed with transplant and the effects of skeletal muscle disuse can be investigated. The skeletal muscle dysfunction may be due to disuse, medication or associated disease. The combined effects of these on Na^+, K^+-ATPase and plasma $[K^+]$ regulation were investigated in this chapter.

7.1 Methods

7.1.1 Subjects, Experimental overview

Eight thoracic transplant patients (LTx) and eight age- and sex-matched healthy controls (CON) participated in the study. LTx were studied in collaboration with the Department of Respiratory Medicine, and The Heart & Lung Transplant Service, The Alfred

Hospital, Melbourne, Victoria. All protocols and procedures were approved by Victoria University of Technology Human Research Ethics Committee and The Alfred Group of Hospitals Ethics Committee. LTx subjects in this study participated in 3 tests, comprising anthropometric measurements, a resting muscle biopsy and an incremental exercise test with arterialised-venous blood samples. Due to ethical considerations, all testing of patients was conducted at the Alfred Hospital, whereas CON were tested in the Human Performance Laboratory at Victoria University, using the same equipment and methods as subjects in Chapter 5 and 6. All measures taken at the two locations were compared and validated with no significant differences seen between workrates on ergometers, $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, and blood analyses of [Hb], Hct, pO_2, PCO_2, plasma [lactate], $[H^+]$ and $[K^+]$. Validation data appears in Appendix B3.

7.1.2 Anthropometric measurements

Age, height, body mass, skinfold thickness, and thigh volumes were determined as detailed in Chapter 3. Lung function measurements were performed as described in Chapter 3 and results were compared to predicted values as shown in Appendix B1.

7.1.3 Resting muscle biopsy

Two resting vastus lateralis muscle biopsies were taken in CON, as detailed in Chapter 3. LTx had two resting vastus lateralis biopsies taken during a routine surveillance bronchoscopy procedure, conducted while patients were sedated with the short-acting anaesthetic benzodiazipine, Midazolam. This procedure is unlikely to effect resting muscle biopsy measures, but the effects are unknown. The treatment of muscle for both groups was described in detail in Chapter 3. Muscle biopsy samples were analysed for 3-*O*-MFPase activity and Na^+, K^+-ATPase content, fibre type proportions and metabolites. Respiratory data were also collected for the incremental exercise tests as described in

Chapter 3. Arterialised venous blood samples were collected each min during the incremental test and analysed for pO_2, pCO_2, [Hb], [Hct], $[H^+]$, plasma [lactate], and $[K^+]$, as previously described (Chapter 3).

7.1.4 Incremental Exercise test

CON performed their incremental exercise test (as described in Chapter 3) in the Human Performance Laboratory at Victoria University of Technology. Patients performed an incremental test on an electrically braked cycle ergometer (Siemans-Elema AB, Sweden) at The Alfred Hospital. Patients increased the workrate by 16W each minute during the incremental test until volitional exhaustion. Expired air was measured for $\dot{V}_E$, $\dot{V}CO_2$, and $\dot{V}O_2$ using a SensorMedics metabolic measurement cart interfaced with an IBM compatible computer.

7.2 Results

Physical characteristics (Chapter 3) did not differ significantly between CON and LTx. The mean (±SD) duration post-transplant was 13.5 ± 8.7 months, ranging from 3-24 months after receiving heart /lung (HLTx, n = 2), single-lung (SLTx, n = 2) or double-lung (DLTx, n = 4) transplants. The diagnosis, operation and medication records of the recipients are shown in Table 3.3 (Chapter 3). No control subjects reported taking any medication.

7.2.1 Muscle Na^+,K^+-ATPase function

7.2.1.1 Maximal K^+-stimulated 3-O-MFPase Activity

The maximal *in-vitro* K^+ stimulated 3-*O*-MFPase activity in skeletal muscle was 31% higher in LTx than in CON when expressed per gram wet weight ($P < 0.05$, Table 7.1). However this difference was not significant (17.0%), when expressed relative to muscle protein content ($P = 0.13$, Table 7.1). There was no difference in muscle protein content between LTx and CON (Table 7.2).

Table 7.1 Maximal K^+ stimulated 3-*O*-MFPase activity in LTx and CON

	3-*O*-MFPase activity (nmol min^{-1} g^{-1} wet wt.)		**3-*O*-MFPase activity (pmol min^{-1} mg^{-1} protein)**	
Subject	**LTx**	**CON**	**LTx**	**CON**
1	172	187	1011	1041
2	304	197	1785	1528
3	226	149	1677	1652
4	200	146	1129	1184
5	261	161	1710	1115
6	199	129	1101	962
7	194	203	1493	1172
8	203	170	1620	1095
Mean ±SEM	220 ± 15*	168 ± 9	1426 ± 110	1219 ± 86

* LTx > CON ($P < 0.05$)

Table 7.2 Muscle protein content (mg.100mg^{-1}muscle wet wt) in LTx and CON

	Protein	
Subject	**LTx**	**CON**
1	16.98	17.92
2	17.01	12.92
3	13.48	9.02
4	17.71	12.32
5	15.29	14.46
6	18.10	13.41
7	13.00	17.36
8	12.51	15.56
Mean ±SEM	15.5 ± 0.8	14.9 ± 1.0

7.2.1.2 Na^+,K^+-ATPase content

Na^+,K^+-ATPase content, as determined from the [^{3}H]-ouabain binding site concentration, did not differ between the LTx and CON (P = 0.32, Table 7.3).

Table 7.3 Na^+,K^+-ATPase content (pmol.g^{-1} wet wt.) as determined from the [^{3}H]-ouabain binding site concentration in LTx and CON

	Na^+,K^+-ATPase content	
Subject	**LTx**	**CON**
1	264	277
2	229	330
3	305	209
4	265	207
5	225	191
6	214	219
7	395	263
8	334	302
Mean ±SEM	279 ± 22	250 ± 18

7.2.1.3 Skeletal muscle fibre types and metabolites

LTx exhibited a higher proportion of type II fibres than CON ($P < 0.05$, Figure 7.1) Resting skeletal muscle metabolites are shown in Table 7.4. LTx had significantly lower resting muscle content of ATP, AMP and higher content of IMP, Lactate and [H^+], ($P < 0.05$).

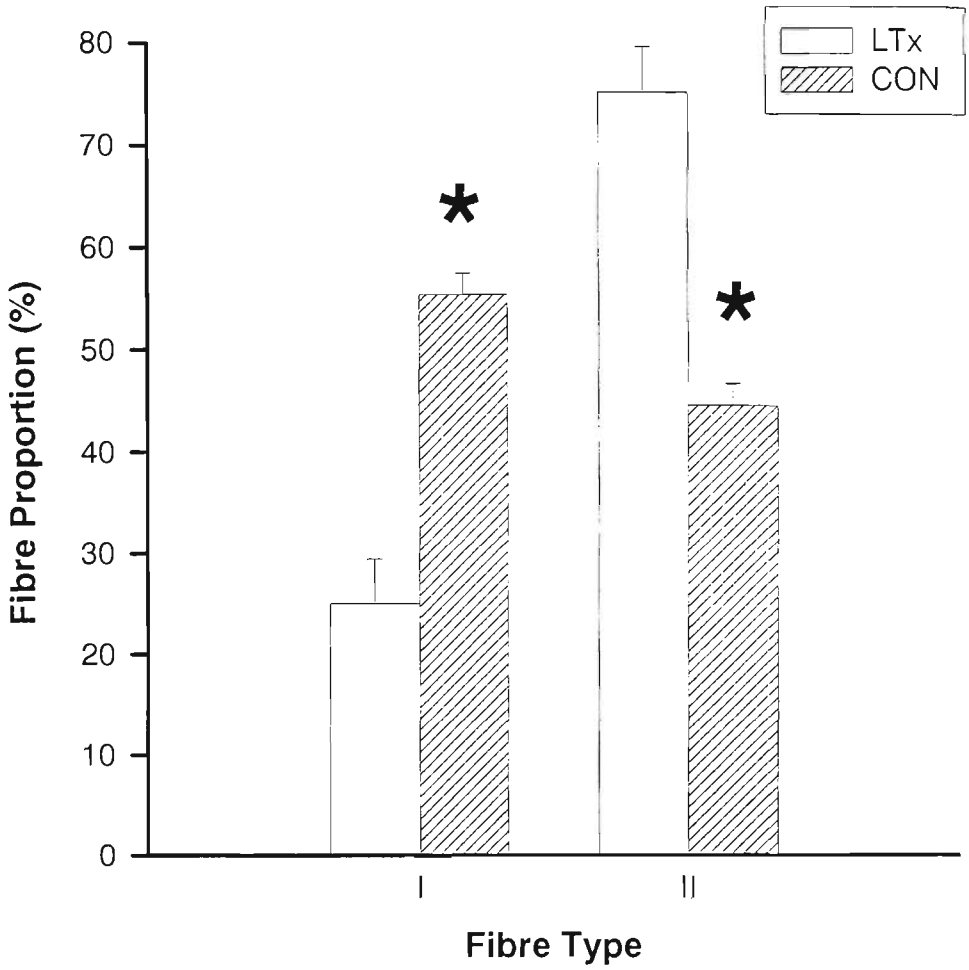

Figure 7.1 Skeletal muscle fibre type proportions in LTx and CON. Mean ± SEM n=7, * LTx different to CON.

Table 7.4 Resting skeletal muscle metabolites

	LTx	CON
ATP	21.41 ± 1.16*	25.97 ± 1.3
ADP	2.81 ± 0.19	2.41 ± 0.14
AMP	0.12 ± 0.02*	0.24 ± 0.06
IMP	0.26 ± 0.04*	0.05 ± 0.01
Cr	55.02 ± 4.48	46.88 ± 4.01
PCr	96.81 ± 4.96	93.18 ± 2.67
Lactate	16.26 ± 0.96*	8.40 ± 0.86
$[H^+]$	86.8 ± 2.6*	77.4 ± 1.3
Glycogen	400 ± 80	462 ± 35

Values are expressed as mmol.kg^{-1} dry weight of muscle, except H^+ (nmol.L^{-1}), and glycogen (mmol glucosyl units.kg^{-1} dry muscle), n=8, Mean ± SEM. *LTx significantly different to CON ($P < 0.05$)

7.2.2 Relationship between skeletal muscle 3-*O*-MFPase activity, Na^+, K^+-ATPase content, metabolites, and drug treatment

There were no relationships between resting 3-*O*-MFPase activity, Na^+, K^+-ATPase content and resting skeletal muscle metabolites. 3-*O*-MFPase activity was not correlated to $\dot{V}O_2$ peak in these subjects. There were no clear relationships between 3-*O*-MFPase activity or Na^+, K^+-ATPase content and the drug treatment that all patients received, Cyclosporine, Azathioprine, and Prednisilone.

7.3 Resting HR and blood data

Resting HR and blood data are shown in table 7.5. Resting heart rate, and pO_2 were not significantly different than CON. Adequate arterialisation was shown in both groups and the LTx displayed a 12% higher pCO_2 (Table 7.5). LTx were slightly anaemic in comparison to CON with [Hb] and Hct 16.3% and 5.3% lower respectively ($P < 0.05$). Resting plasma [lactate] was 56% higher in LTx in comparison to CON, $P < 0.05$, while plasma $[H^+]$ was lower at rest in CON ($P < 0.05$, Table 7.5). Arterialised-venous plasma $[K^+]$ was 28% higher at rest (LTx 4.85 ± 0.14 vs CON 3.80 ± 0.07 $mmol.l^{-1}$).

Table 7.5 Resting HR and Blood data for LTX and CON.

	LTx	**CON**
HR ($b.min^{-1}$)	88 ± 5	76 ± 3
Haemoglobin ($g.dl^{-1}$)	11.6 ± 0.3*	13.8 ± 0.3
Haematocrit (%)	34.5 ± 0.8*	39.8 ± 0.5
pO_2 (mmHg)	78.1 ± 3.2	71.9 ± 2.9
pCO_2 (mmHg)	38.5 ± 1.0*	34.4 ± 1.3
$[H^+]$ ($nmol.l^{-1}$)	37.9 ± 1.0*	36.7 ± 0.3
[lactate] ($mmol.l^{-1}$)	1.55 ± 0.23*	0.99 ± 0.11
$[K^+]$ ($mmol.l^{-1}$)	4.85 ± 0.14*	3.80 ± 0.07

n=8,mean ± SEM, *LTx significantly different to CON ($P < 0.05$)

7.3.1 Cardiorespiratory measures during incremental exercise

The LTx performed significantly worse during incremental exercise (Table 7.6). The peak work rate and $\dot{V}O_2$ peak achieved by LTx were only 40% and 49% of that attained by CON (Figure 7.2). Peak HR and peak $\dot{V}_E$ were 76% and 58% of CON (Figure 7.2, Table 7.6).

Table 7.6 Peak data during incremental exercise test

	LTx	**CON**
Workrate (W)	82 ± 11*	222 ± 28
Total work (kJ)	16.17 ± 3.32*	72.34 ± 17.33
$\dot{V}O_2$ ($l.min^{-1}$)	1.21 ± 0.08*	2.46 ± 0.34
$\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$)	18.8 ± 1.5*	35.7 ± 2.4
$\dot{V}CO_2$ ($l.min^{-1}$)	1.51 ± 0.16*	2.96 ± 0.37
$\dot{V}_E$ (l)	64.0 ± 6.1*	110.7 ± 13.7
RER	1.23 ± 0.08	1.21 ± 0.04
HR peak ($b.min^{-1}$)	136 ± 5*	178 ± 5

Mean ± SEM, n = 8, RER = respiratory exchange ratio, *LTx <CON ($P < 0.05$)

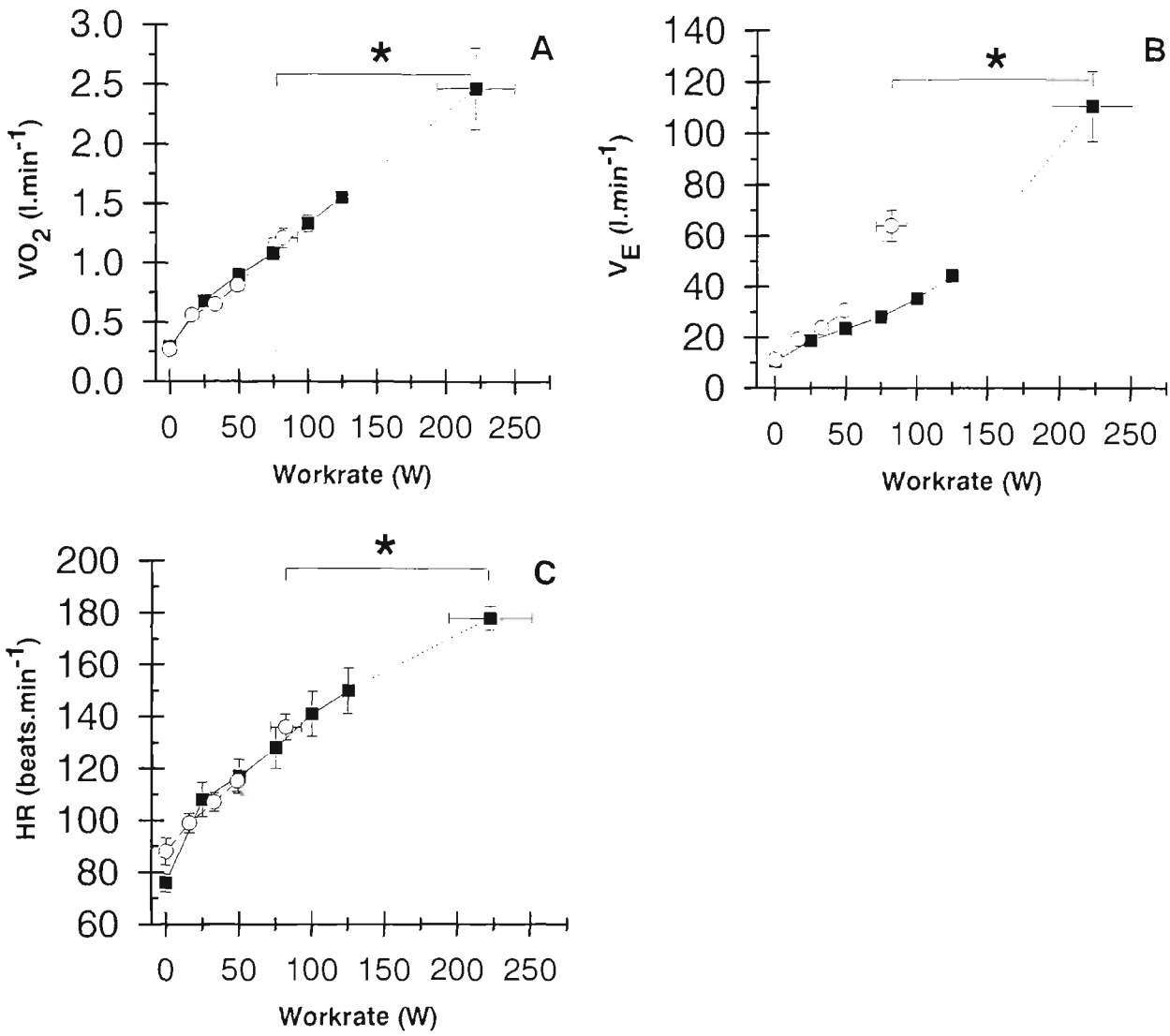

Figure 7.2 Pulmonary $\dot{V}O_2$, $\dot{V}_E$, and Heart Rate at rest, during submaximal workrates and at peak exercise during incremental exercise for (○) LTx and (■) CON. Horizontal error bars for peak values indicate mean and sem of peak workrate. Data: n=8, Mean ± SEM. * peak values for LTx < CON ($P < 0.05$)

7.3.2 Blood measures during incremental exercise

7.3.2.1 Changes in plasma volume

Plasma volume did not differ significantly from resting levels during exercise or recovery for LTx, while CON plasma volume decreased during exercise and had not returned to resting levels by 30 min post exercise. The change in plasma volume was significantly greater in CON at peak exercise and remained lower until 20 min post exercise (Figure 7.3).

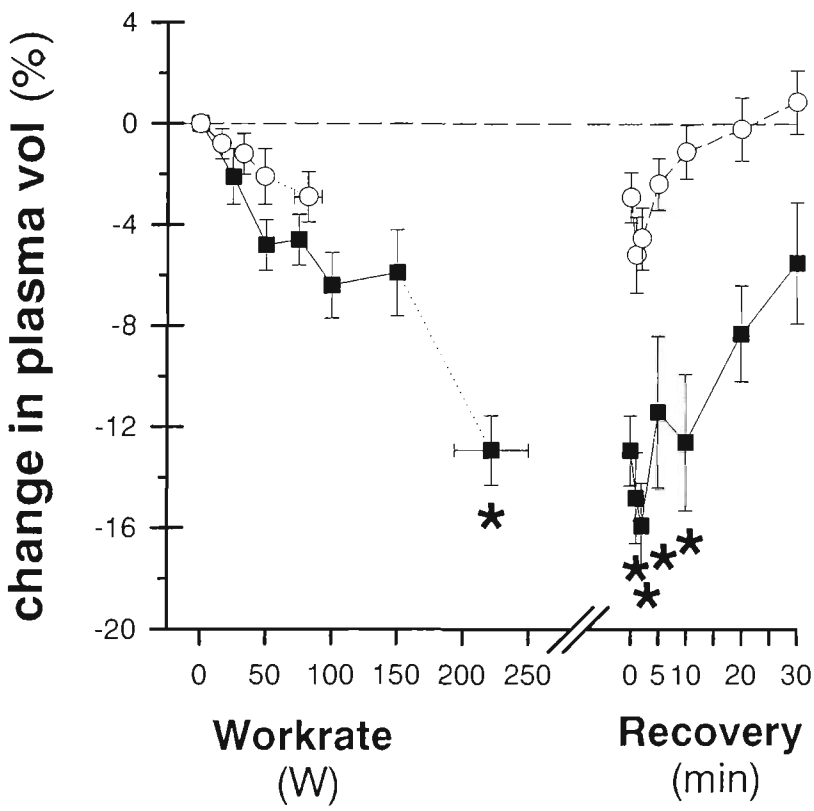

Figure 7.3 Calculated percent change in plasma volume from rest, during submaximal workrates and at peak exercise during incremental exercise for (○) LTx and (■) CON. Horizontal error bars for peak values indicate mean and sem of peak workrate. The zero recovery timepoint is the peak exercise value replotted. Data: n=8, Mean ± SEM. * CON lower than LTx ($P < 0.05$).

7.3.2.2 Plasma [lactate] and [H^+] during incremental exercise

Plasma [H^+] and [lactate] are plotted against absolute workrate (Figure 7.4A,B) and against relative workrate (% $\dot{V}O_2$ peak, Figure 7.4C,D). The peak exercise [H^+] for CON was not significantly higher than LTx but [H^+] peaked in recovery at 5 min post and this remained higher than LTx. CON [H^+] had returned to within resting levels by 30 min post (Figure 7.4A). Peak plasma lactate was higher in CON (9.40 ± 1.22 mmol.l^{-1}) than LTx (5.75 ± 0.50 mmol.l^{-1} $P < 0.05$) and was significantly higher in CON from 2-10 min post exercise (Figure 7.4B). The submaximal plasma [H^+] and [lactate] were similar for LTx and CON when compared at a similar relative intensity (Figure 7.4C,D).

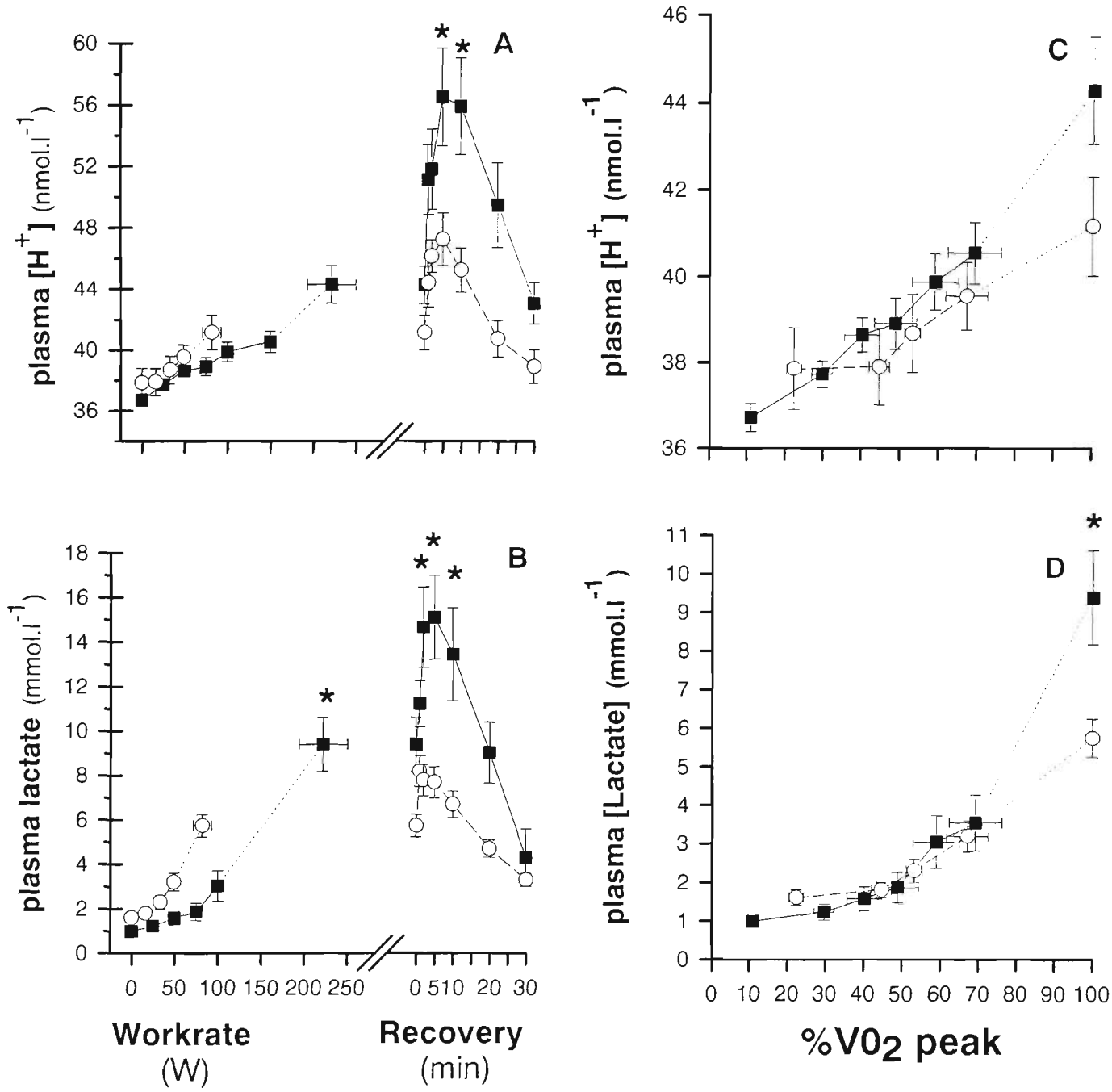

Figure 7.4 (**A**) Plasma [H^+] and (**B**) plasma [Lactate] at rest, during incremental exercise and during 30 min of recovery for (○) LTx and (■) CON. Horizontal error bars for peak values indicate mean and sem of peak workrate. The zero recovery timepoint is the peak exercise value replotted. (**C**) Plasma [H^+] and (**D**) plasma [lactate] plotted against percent $\dot{V}O_2$ peak Data: n=8, Mean ± SEM. * CON > LTx, ($P < 0.05$).

7.3.2.3 Plasma [K^+] response during exercise

Arterialised-venous plasma [K^+] increased with exercise ($P < 0.05$ Figure 7.5), with peak plasma [K^+] similar in both groups (LTx 5.93 ± 0.12 vs CON 5.86 ± 0.24 mmol.l^{-1}) despite a greater than two-fold higher workrate in CON. The plasma [K^+] fell in recovery but remained higher in LTx than in CON at all times ($P < 0.05$). Submaximal plasma [K^+] could not be directly compared as LTx and CON performed 16W and 25W increments respectively. However plasma [K^+] was plotted against percent $\dot{V}O_2$ peak (Figure 7.6A). This shows LTx plasma [K^+] was clearly greater than CON at low relative workrates, converging at 100% $\dot{V}O_2$ peak. As the change in [K^+] is dependent on the amount of work done, the Δ[K^+] and Δ[K^+] work^{-1} ratio were also examined. The peak rise in [K^+] during exercise (Δ[K^+]) was less ($P < 0.05$) in LTx (1.08 ± 0.15*vs* 2.06 ± 0.19, Figure 7.6B,C), and the Δ[K^+] work^{-1} ratio at peak exercise was greater than 2-fold higher in LTx (84.5 ± 15.9 *vs* 37.1 ± 5.9 nmol.l^{-1}.J^{-1}, Figure 7.6D, $P < 0.05$).

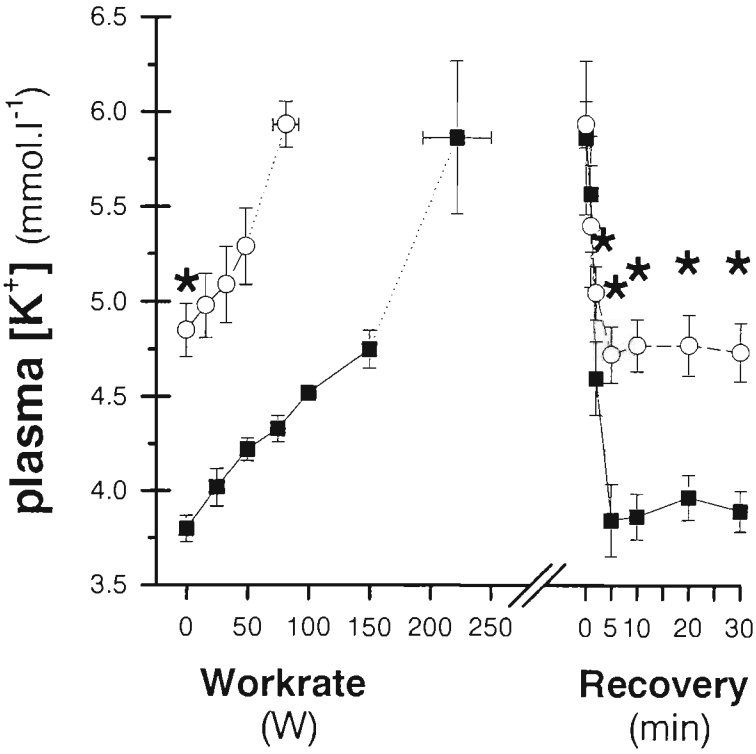

Figure 7.5 Plasma [K^+] at rest, during incremental exercise and during 30 min of recovery for (○) LTx and (■) CON. Horizontal error bars for peak values indicate mean and sem of peak workrate. The zero recovery timepoint is the peak exercise value replotted. Data: n=8, Mean ± SEM. * LTx > CON ($P < 0.05$).

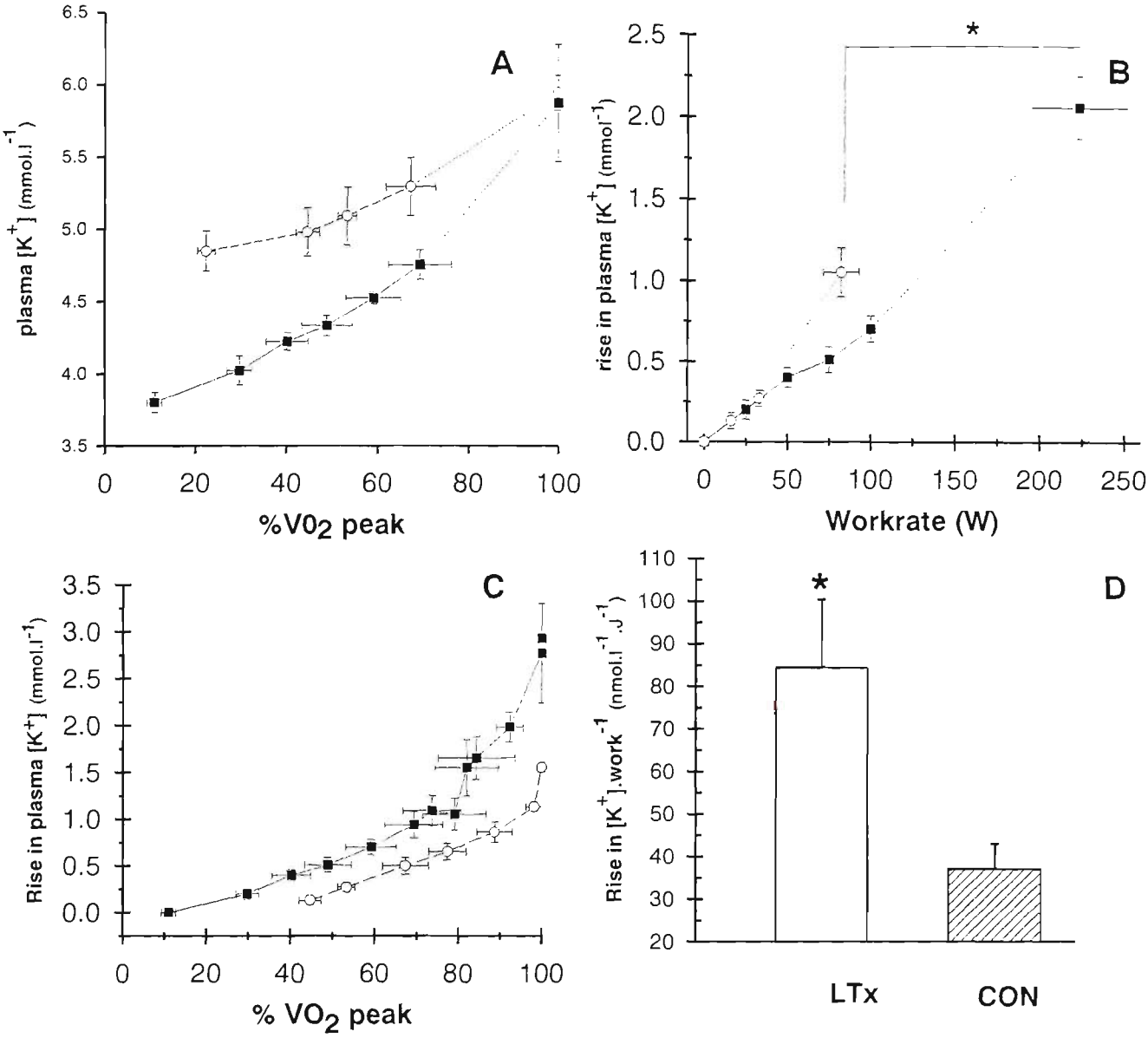

Figure 7.6 (**A**) Plasma [K^+] against percent $\dot{V}O_2$ peak (**B**) rise in plasma [K^+] against (**B**) workload and (**C**) percent $\dot{V}O_2$ peak rise for (○) LTx and (■) CON. Horizontal error bars for submax values indicate mean and sem of % $\dot{V}O_2$ peak. (**D**) $\Delta[K^+]$ $work^{-1}$ ratio for LTx and CON. Data: n=8, Mean ± SEM. *LTx < CON, P < 0.05.

7.3.2.4 Post-exercise decline in plasma [K^+]

There were no significant differences in the rate of decline in [K^+] from peak exercise to 1 min recovery for LTx and CON while the rate of decline from peak to 2 min tended (P=0.06) to be slower in LTx. This rate was faster at 5 min recovery in CON compared to LTx (P < 0.05, Table 7.7).

Table 7.7 Decline in $[K^+]$ from peak exercise to 1, 2 and 5 min recovery. Mean ± SEM

Recovery	$\Delta[K^+]_{recovery}$ **(mmol.l^{-1}.min^{-1})**	
	LTx	**CON**
+1 min (n=4)	0.40 ± 0.26	0.51 ± 0.08
+2 min (n =8)	0.44 ± 0.07	0.63 ± 0.06
+5 min (n =8)	0.24 ± 0.03*	0.40± 0.03

Mean ± SEM, n = 8, *LTx <CON ($P < 0.05$)

7.3.3 Relationship between 3-*O*-MFPase activity, Na^+,K^+-ATPase content, and $[K^+]$ during exercise

The maximal *in vitro* 3-*O*-MFPase activity in skeletal muscle was not related to plasma $[K^+]$ variables during the incremental exercise for LTx and CON. $\dot{V}O_2$ peak was positively correlated to ΔK^+ but inversely related to the $\Delta[K^+]$.work^{-1} ratio (Figure 7.7 A,B).

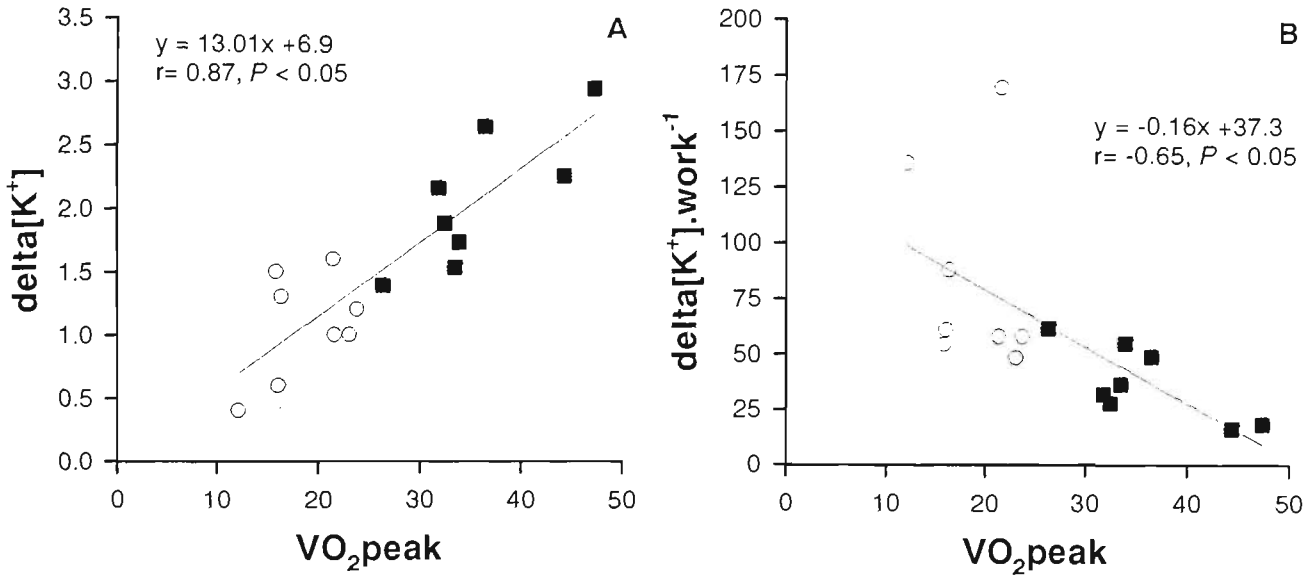

Figure 7.7 The relationships between $\dot{V}O_2$ peak ($ml.kg^{-1}.min^{-1}$) and **A)** $\Delta[K^+]$ ($mmol.l^{-1}$) and **(B)** $\Delta[K^+].work^{-1}$ ($nmol.l^{-1}.J^{-1}$) for (○) LTx and (■) CON, n=16.

7.4 Discussion

7.4.1 Na^+, K^+-ATPase function in transplant patients

The most important finding from this study was the dissociation between muscle Na^+, K^+-ATPase and markers of abnormal plasma $[K^+]$ response during exercise. Patients demonstrated an increased $[K^+]$ at rest, increased $\Delta[K^+].work^{-1}$, but did not show a compromised Na^+, K^+-ATPase content or activity in resting muscle compared to age-sex matched controls. The increased $\Delta[K^+].work^{-1}$ confirm the results for transplant patients who had grossly impaired plasma $[K^+]$ response during incremental exercise (Hall et al. 1994) while the lack of downregulation of Na^+, K^+-ATPase content opposes the findings in heart failure patients (Barlow et al. 1998; Nørgaard et al. 1990). Adding this to the finding of a lack of a correlation between Na^+, K^+-ATPase and plasma $[K^+]$ response, it could be concluded that factors in addition to Na^+, K^+-ATPase total content and activity are important in regulating plasma $[K^+]$. This is supported by the finding by Kjeldsen et

al. (1990a) that endurance training reduced exercise-induced hyperkalemia despite no change in Na^+, K^+-ATPase content.

An alternative explanation to the above finding could be the technique used to measure Na^+, K^+-ATPase activity. This technique measured maximal *in vitro* K^+ stimulated 3-*O*-MFPase activity *in vitro,* where it is possible that that this maximal activity as well as Na^+, K^+-ATPase content are maintained in the transplant patients but that *in vivo* the electrical, ionic or hormonal activation of the Na^+, K^+-ATPase is compromised.

The transplant patients had severe metabolic abnormalities at rest with a reduction in muscle ATP content as well as elevated IMP and lactate content. It has been suggested that local metabolic supply could affect Na^+, K^+-ATPase function (James et al. 1996; Körge 1998). If these effects are exacerbated during exercise. then it is possible that *in vivo* Na^+, K^+-ATPase activity may be compromised, as opposed to the *in vitro* 3-*O*-MFPase assay where energy supply is not limiting. Of note is the fact that the transplant patients had a higher resting muscle $[H^+]$. This finding is supported by the ^{31}P-MRS study of Evans et al. (1997) in which transplant patients' quadriceps muscle intracellular $[H^+]$ was higher and increased earlier during incremental exercise compared to controls. This H^+ disturbance may again influence *in vivo* Na^+, K^+-ATPase activity (Huang & Askari 1984) but would not be detected in the *in vitro* 3-*O*-MFPase assay used in this study, unless it caused a structural change in the Na^+, K^+-ATPase enzyme.

7.4.1.1 Chronic disuse and disease effects on Na^+, K^+-ATPase

Lung transplant patients are characterised by chronic inactivity prior to transplant and inactivity due to limb immobilisation or disease states and these have been associated with a downregulation in Na^+, K^+-ATPase content (Jebens et al. 1995; Kjeldsen et al. 1986; Leivseth et al. 1992, 1994; Nørgaard et al. 1990). Nørgaard et al. (1990) showed a

25% reduction in Na^+, K^+-ATPase content in patients with congestive heart failure, while Leivseth et al. (1994) showed that Na^+, K^+-ATPase content was decreased by 27% in the deltoid muscle of patients with shoulder impingement. Druml et al. (1988) showed that while basal Na^+, K^+-ATPase activity was decreased by 30% in skeletal muscle of rats with uraemic chronic renal failure, Na^+, K^+-ATPase content was not affected. They concluded that Na^+, K^+-ATPase turnover rate was decreased. The surprising result of the present study is that our patient group did not display the expected decline in Na^+, K^+-ATPase activity or content seen with the above disease states. Possible reasons for this discrepancy include pre-existing disease alterations in Na^+, K^+-ATPase and drug treatment such as glucocorticoid administration.

Hypertension

Six of the eight patients were hypertensive, a condition known to increase soleus muscle Na^+, K^+-ATPase content in spontaneously hypertensive rats (Pickar et al. 1994). Our patients displayed increased Na^+, K^+-ATPase activity compared to controls, but this did not show a significant difference in Na^+, K^+-ATPase content compared to controls.

Cystic Fibrosis

Two of the eight transplant patient suffered from cystic fibrosis and this has been shown to alter Na^+, K^+-ATPase function in epithelial cells and erythrocytes (Luczay et al. 1997; Resnik et al. 1986). Na^+, K^+-ATPase activity in human bronchial epithelium was two-fold higher in cystic fibrosis patients than controls (Peckham et al. 1997). Reznik et al. (1986) noted that the affinity of the Na^+, K^+-ATPase for K^+ was significantly lower in erythrocytes of cystic fibrosis patient's compared to controls. If this phenomenon also occurred in the skeletal muscle of these patients it could help to partially explain the higher Na^+, K^+-ATPase content but impaired K^+ regulation in at least the two cystic fibrosis patients. These two patients had the highest Na^+, K^+-ATPase content (but not

Na^+, K^+ ATPase activity) of the patient group but did not appear to differ from the other patients with respect to their plasma [K^+] response to exercise.

7.4.1.2 Glucocorticoids

Glucocorticoids are commonly administered to transplant patients and often result in myopathy and muscle weakness (Ruff et al. 1982; Seene 1994). Ruff et al. (1982) showed that glucocorticoid-induced EDL muscle atrophy did not produce muscle weakness by impairing sarcolemmal excitability or excitation-contraction coupling. Further, glucocorticoid (dexamethasone) treatment has been shown to increase skeletal muscle Na^+, K^+-ATPase content by 31% in chronic obstructive lung disease patients (Ravn & Dørup 1997), with similar results shown in rat skeletal muscle (Dørup & Clausen 1997). This may partly explain why Na^+, K^+-ATPase content was normal and Na^+, K^+-ATPase activity increased in LTx. It is possible that this glucocorticoid-induced rise in Na^+, K^+-ATPase content may not subsequently invoke or maintain normal [K^+] handling during and after exercise.

7.4.2 Altered muscle fibre types in transplant patients

Human vastus lateralis typically contains an even distribution of type I and type II muscle fibres (Saltin & Gollnick 1983). Lung transplant patients suffer from many years of muscle disuse and in this study lung transplant patients displayed a greater proportion of type II and a lower proportion of type I fibres. High proportions of Type II fibres have been reported in a variety of patients including chronic obstructive pulmonary disease patients (Jakobsson et al.1990; Satta et al. 1997), heart failure patients (Sullivan et al. 1990), and heart transplant recipients (Bussieres et al. 1997). All these patients suffer from chronic disuse and this is probably the most important factor underlying their

muscle fibre type abnormalities. It has been documented that skeletal muscle is adaptable to functional demand (see 2.10.1, Chapter 2). There is good evidence of a slow to fast fibre transformation in animal models with disuse (Gardiner et al. 1992), while the data for humans is not as extensive (Häggmark & Eriksson 1979; Halkjær-Kristensen & Ingemann-Hansen 1985; Satta et al. 1997; Sargeant et al. 1977).

7.4.2.1 Effect of altered muscle fibre type on Na^+, K^+-ATPase

The altered muscle fibre type seen in the transplant patients could possibly help to explain increased Na^+, K^+-ATPase activity seen in comparison to matched controls. Most studies showed human skeletal muscle Na^+, K^+-ATPase does not display a fibre type dependence (Benders et al. 1992; Dørup et al. 1988; Nørgaard 1986) but Chin & Green (1993) indicated that a relationship between Na^+, K^+-ATPase content and oxidative potential in rat skeletal muscle. There is evidence for fibre type dependence in rat skeletal muscle, with fast twitch EDL muscle having 20-30% higher Na^+, K^+-ATPase content than soleus (Clausen et al. 1982; Nørgaard 1986). However it is inappropriate to relate fibre type with Na^+, K^+-ATPase activity in the LTx group as other factors also influence Na^+, K^+-ATPase activity. These include medication and pre-existing diseases (Pickar et al 1994; Ravn & Dørup 1997; Reznik et al 1986).

7.4.3 Other factors influencing low exercise capacity in LTx

As shown by others (Ambrosino et al. 1996; Banner et al.1989; Theodore et al. 1987) the transplant patients' peak oxygen consumption was only 49% of the healthy control value. In the present study, the transplant patients as a group, demonstrated a mild mixed obstructive/restrictive ventilatory defect (as assessed by spirometry, Crapo et al. 1981; Hall et al. 1994; Williams et al. 1992). Only one of the eight transplant recipients

reached ventilatory limitation during exercise and no patients exhibited desaturation. It could be argued that the low HR peak in these patients largely influenced the low cardiac output seen, but the limitation has been attributed to early exercise cessation due to peripheral factors reducing muscle O_2 utilisation (Schwaiblmair et al. 1999; Williams et al 1992). This suggests that peripheral factors were primarily responsible for the severe exercise limitation observed in these patients. It has previously been stated that transplantation removes the cardiac or pulmonary limitation to exercise (Schwaiblmair et al. 1999; Williams et al. 1992). Therefore iatrogenic and/or deconditioning factors must be important factors for the persistent exercise limitation post transplantation (Hall et al. 1994; Pantoja et al. 1999; Schwaiblmair et al. 1999; Williams et al. 1992).

7.4.3.1 Iatrogenic effects

Despite the lack of a relationship between the medications that patients in this study received and Na^+, K^+-ATPase content or activity, other studies have indicated that these medications may alter skeletal muscle function including Na^+, K^+-ATPase function.

7.4.3.2 Cyclosporine

Cyclosporine (CsA) is known to cause hyperkalemia due to either aldosterone deficiency or distal renal tubular dysfunction (Olyaei et al. 1999). All LTx received CsA and hence, this most likely explains the elevated resting $[K^+]$ in the transplant patients. No studies have investigated the effect of CsA on skeletal muscle Na^+, K^+-ATPase but interestingly, clinical does of CsA were correlated with increased Na^+, K^+-ATPase activity in erythrocytes of kidney transplant patients (Ferrer-Martínez et al. 1999). Although the mechanism whereby this might occur has not been elucidated, if a similar effect occurred in skeletal muscle, then CsA might help to explain the maintained Na^+, K^+-ATPase content and activity.

An adverse effect of CsA is inhibition of mitochondrial respiration *in vitro* (Hokanson et al. 1995; Mercier et al. 1995). In a collaborative study with the patients used in this thesis, the rates of mitochondrial ATP production and mitochondrial enzyme activities were found to be markedly reduced in comparison to matched controls (Wang et al. 1999). This suggests that the skeletal muscle of LTx have energetic limitations.

7.4.4 Conclusion

The finding of a maintained or increased Na^+, K^+-ATPase activity and content in lung transplant patients was surprising given the chronic inactivity and the impaired plasma $[K^+]$ response seen in this group. Factors such as pre-existing medical conditions or drug treatment (glucocorticoids), may increase Na^+, K^+-ATPase activity and content without necessarily inferring improved $[K^+]$ handling during and after exercise. Further studies are required investigating the effect of these drugs, pre-existing disease states and their impact on Na^+, K^+-ATPase function, $[K^+]$ regulation and exercise capacity in these patients.

Chapter 8 General Summary

8.0 Introduction

Skeletal muscle Na^+, K^+-ATPase content and activity are vital to the preservation of membrane excitability and have been implicated in several physiological processes, including muscle fatigue. In this thesis, the first study involved the modification of a sensitive *in vitro* measure of maximal Na^+, K^+-ATPase activity. The three studies examined factors that affect muscle Na^+, K^+-ATPase content and activity and plasma $[K^+]$, including acute exercise, chronic training, and muscle disuse associated with severe disease culminating in lung transplantation. These studies enabled further exploration of the role of Na^+, K^+-ATPase in regulating the plasma $[K^+]$ response during exercise in humans.

8.1 Major Findings

8.1.1 Modification of 3-*O*-MFPase assay for measurement of human skeletal muscle Na^+, K^+-ATPase activity

The first study modified the K^+-stimulated 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase) assay for measurement of Na^+, K^+-ATPase activity in human skeletal muscle. Assay specificity for the Na^+, K^+-ATPase was confirmed, while homogenate treatment and ligand concentration were all optimised. The maximal K^+-stimulated 3-*O*-MFPase activity in quadriceps muscle homogenates in seven healthy untrained subjects was (mean±SE) 292±10 nmol min^{-1} g^{-1} wet wt (1745±84 pmol min^{-1} mg^{-1} protein). These

modifications optimised the 3-*O*-MFPase assay allowing valid, reliable measurements of Na^+,K^+-ATPase activity in small samples of human skeletal muscle.

8.1.2 Fatiguing exercise depresses maximal Na^+, K^+-ATPase activity in human skeletal muscle and training does not attenuate this

The first hypothesis of this thesis was supported: acute fatiguing exercise of short duration (<2 min) reduced peak knee extensor torque by 49.5±2.8 % and also depressed Na^+, K^+-ATPase activity by 13.5 %. This is the first report of a depressed Na^+, K^+-ATPase activity with fatiguing exercise in any species. The second hypothesis was not supported as there was no significant effect of endurance training or resistance training on 3-*O*-MFPase activity in resting muscle. However the endurance trained subjects did show a 17% higher Na^+, K^+-ATPase content than untrained controls. The third hypothesis was not supported as the fatigue-induced decline in Na^+, K^+-ATPase activity was not attenuated with training. The exact mechanisms underlying the acute impairment in Na^+, K^+-ATPase activity as well as possible training effects in minimising these agents' effects on Na^+, K^+-ATPase remains to be determined.

There were inconsistent relationships between Na^+, K^+-ATPase activity and muscle metabolites and these relationships most likely reflect parallel depressive processes as a result of exercise, rather than causal relationships. Due to the *in vitro* nature of the 3-*O*-MFPase assay, structural alterations in the Na^+, K^+-ATPase enzyme are the most likely reason for the measured fatigue-induced depression in 3-*O*-MFPase activity. However it is likely that metabolic perturbations may further exacerbate these changes *in-vivo*. It is possible to speculate that the likely agents causing these structural alterations with fatigue include reactive oxygen species and raised intracellular [Ca^{2+}].

8.1.3 Excessive Plasma [K^+] responses to exercise are higher in lung transplant patients, despite normal Na^+, K^+-ATPase activity and content

Despite the removal of the ventilatory limitation to exercise in the lung transplant patients by transplantation, they still exhibited severely limited exercise capacity. Lung transplant patients also showed an abnormally high Type II fibre proportion, consistent with chronic muscle disuse. The fourth hypothesis was not supported. An elevated plasma [K^+] response was seen during incremental exercise in lung transplant patients evidenced by an excessive rise in the plasma [K^+].work^{-1} ratio during exercise. This was not explained by a lower Na^+, K^+-ATPase content or activity since these were not significantly different from age-matched controls. Thus, an abnormal Na^+, K^+-ATPase activation, or reduced extracellular K^+ clearance is indicated in the excessive hyperkalemia displayed in these patients.

8.1.4 Relationships between Na^+, K^+-ATPase activity and content, fibre type, $\dot{V}O_2$ peak and age for all healthy subjects

All the healthy subjects, comprising untrained, endurance trained, resistance trained (Chapter 6), and age-matched controls (Chapter 7) were pooled to investigate the relationships between Na^+, K^+-ATPase activity and content, plasma [K^+] and muscle function. As discussed in Chapter 6, Na^+, K^+-ATPase activity correlated with Na^+, K^+-ATPase content when all four healthy groups were analysed ($r = 0.61$, $P < 0.05$, Figure 8.1). This relationship helps validate the modified 3-*O*-MFPase assay against the most widely accepted method for determining Na^+, K^+-ATPase content ([^{3}H]-ouabain binding). The moderate correlation is likely to be influenced by the variability in each Na^+, K^+-ATPase activity measure of around 5% and [^{3}H]-ouabain binding of around 5-10%,

which increases the scatter of the data. Na^+, K^+-ATPase activity also correlated with $\dot{V}O_2$ peak (r = 0.46, $P < 0.05$), indicating a link with physical activity and thus an important role of Na^+, K^+-ATPase in skeletal muscle function. This relationship and the stronger correlation between Na^+, K^+-ATPase content and $\dot{V}O_2$ peak (r = 0.70, $P < 0.05$) confirm the relationship shown by Evertsen et al. (1997) and Green et al. (1999a) between these variables. A weak relationship between Na^+, K^+-ATPase activity and the proportion of Type I fibres (r = 0.40, $P < 0.05$) was also evident. Age was also investigated but no relationships were found for any of the above variables.

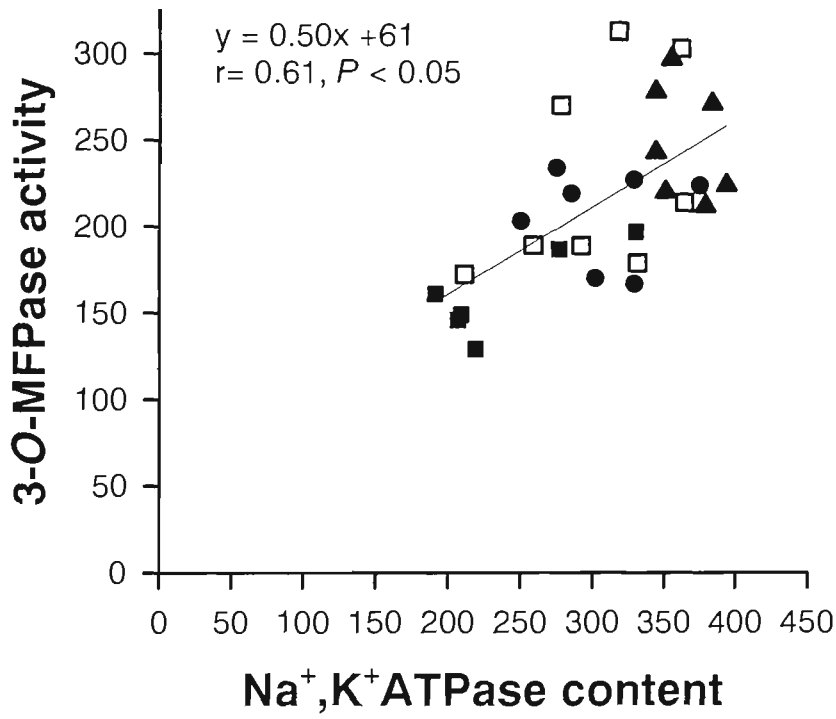

Figure 8.1 The relationship between Na+, K+-ATPase content (pmol.g-1 wet wt) and 3-O-MFPase activity (nmol min-1 g-1 wet wt) untrained (UT, ●), resistance trained (RT, □), endurance trained (ET, σ), and age-matched controls (CON, ν), n=28.

8.1.5 Relationships of Na^+, K^+-ATPase activity and content, with the exercise-induced plasma $[K^+]$ response for all healthy subjects

All the healthy subjects, comprising untrained, endurance trained, resistance trained (Chapter 6), and age-matched controls (Chapter 7) were pooled to investigate the relationships of Na^+, K^+-ATPase activity and content, with plasma $[K^+]$. Variables used to describe the plasma $[K^+]$ response during the $\dot{V}O_2$ peak test included the peak $[K^+]$, $\Delta[K^+]$, and the $\Delta[K^+].work^{-1}$, during exercise as well as the exercise fall in $[K^+]$ 1 and 2 min post exercise. However none of these variables correlated with skeletal muscle Na^+, K^+-ATPase content or activity when a larger muscle muss was sampled. Thus the larger population did not clarify the previous inconsistent relationship between the plasma $[K^+]$ response and Na^+, K^+-ATPase activity or content (Chapter 5). The lack of relationship does not indicate a lack of functional significance of Na^+, K^+-ATPase on circulating K^+ during exercise. Rather it most likely reflects the fact that many other factors influence plasma $[K^+]$, including blood flow to active and inactive muscle, site of blood and muscle sampling, and K^+ clearance rates in active and inactive muscle. The sampling of arterialised-venous blood samples from a dorsal hand vein was a long way removed from the interstitium of contracting muscle. It would clearly be more useful to compare femoral venous $[K^+]$ or quadriceps interstitial $[K^+]$ (Juel et al. 2000b) with v. lateralis Na^+, K^+-ATPase activity, as these sites are more representative of the conditions that the sarcolemmal Na^+, K^+-ATPase is exposed to.

8.2 Recommendations for further research

Studies conducted in this thesis have raised many further questions regarding the effects of acute exercise, training status, chronic inactivity and disease on skeletal muscle Na^+, K^+-ATPase function and $[K^+]$ responses during exercise in humans. Use of a longitudinal experimental design would be of benefit in studies investigating the time course of training effects on Na^+, K^+-ATPase activity and content in both healthy and diseased individuals, as well as the time course of progressive disease effects on Na^+, K^+-ATPase. Relatively little is known about the various subunits of the Na^+, K^+-ATPase enzyme and the different substrate affinities during exercise. Possible alterations in expression of these subunits in response to training, altered muscle fibre type, fatigue and disuse remains to be explored. Intense fatiguing exercise induced a decline in maximal Na^+, K^+-ATPase activity but the mechanisms underling this change remains unknown. Further, the time course of the recovery of this fatigue-induced depression in Na^+, K^+-ATPase activity *in vitro* warrants further investigation. Reactive oxygen species and raised intracellular $[Ca^{2+}]$ are likely to be involved in the proposed Na^+, K^+-ATPase structural alterations and studies on these in relation to muscle fatigue would be appropriate.

The use of histochemical and immunocytochemical methods would be of great benefit to future work on human skeletal muscle. Such work might focus on the expression and location of the various isoforms of the Na^+, K^+-ATPase, including the possibility of translocation, with acute exercise, training and chronic inactivity. The relative lack of studies investigating on Na^+, K^+-ATPase function in human skeletal muscle indicates a great need for further exploration of an important area for research. Research in this area can be expected to have considerable impact on understanding the mechanisms of fatigue and of exercise limitation in sporting and clinical domains, in the prevention or

attenuation of intracellular substructural changes. It is therefore likely that, ultimately, such research will result in enhanced exercise capacity in both healthy and diseased individuals.

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Appendices

Appendix A

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Subject Information Sheet:

Effects of Training on Human Skeletal Muscle Ionic Regulation and Mitochondrial Function

Investigators:
Dr. Michael McKenna ph 9688 4499
Assoc. Prof. Mick Carey* ph 9688 4298
Mr. Steve Fraser ph 9688 4089

Department of Physical Education & Recreation, *Department of Chemistry & Biology, Victoria University of Technology, Footscray, Vic.

Dear

Thankyou for volunteering to participate in this study and for taking the time to read through this information. This study aims to compare muscle size, biochemistry, maximal strength and the development of fatigue during repeated contractions, between strength trained, endurance trained, untrained and a severely untrained patient group. This information sheet describes the nature of the study and the procedures involved for participants. Included are more detailed procedures and consent forms, which you need to complete and bring with you on the first day of testing. You will be required for testing on four occasions, three at the Human Performance Laboratory (Room L305, Building L, V.U.T Footscray campus) and the other test will be conducted at the C.R.E.S.S biomechanics laboratory in the basement of the City campus, 300 Flinders St. Maps are included showing the addresses of both places for testing.
Times that are suitable and the order of testing will be confirmed over the phone by Steve Fraser. Any queries in relation to the study can be directed to Steve Fraser on w:9688 4089 & h:9551 3764 or Dr. Michael McKenna on w:9688 4499, message 9688 4470.

Thankyou once again,
Yours sincerely

Steve Fraser

Please refrain from vigorous exercise, alcohol or tobacco for 24 hours prior to each test. Avoid food for 2 hours prior and caffeine for 4 hours prior to each exercise test.

Day 1: VO_2 peak test with blood sampling. You will be required to attend the Human Performance Laboratory at Footscray to perform a maximal exercise test to exhaustion 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 (detailed procedures for blood sampling are outlined in the paragraph entitled "catheterisation & blood sampling"). Please bring runners, shorts, T shirt and a towel. This test will take approximately 1.5 hours. Shower facilities are available.

Day 2: Anthropometric measurements. At the Human Performance lab a range of non-invasive measurements will be taken including a lung function test where 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. Other tests include underwater weighing and skinfolds for % body fat, as well as leg girth, length and volume measurements. Please bring shorts T shirt, bathers and a towel. This test will take approximately 1 hour. Shower facilities are available.

Day 3: 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 the City Campus, 300 Flinders Street. 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. Please bring shorts, runners and T shirt. This test will take approximately 1 hour.

Day 4. Muscle biopsies and muscle fatigue testing . On the third visit to the Human Performance Laboratory, Footscray Campus, two resting muscle biopsies 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 (detailed procedures for muscle biopsy are outlined in the paragraph entitled "muscle biopsy procedure"). Blood samples will also be taken from a vein in the back of the hand. Please bring shorts, runners and T shirt. This test will take approximately 1.5 hours.

As a volunteer to participate as a subject, you are free to withdraw from the study at any time, without any adverse effects, reactions or discrimination.

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 and Blood Sampling

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 100 ml of blood will be taken during the VO_2max test and 50 ml during the biopsy fatigue 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. 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.

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. A summary report of your results as well as some instant feedback will be given for all tests conducted. Thank you for your co-operation.

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 Training on Human Skeletal Muscle Ionic Regulation and Mitochondrial Function."

being conducted at Victoria University of Technology by :

Dr. Michael J. McKenna, Associate Professor Michael F. Carey

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:

1. Anthropometric measurements, including underwater weighing
2. Lung function measurements
3. Maximal incremental exercise test on a cycle ergometer
4. Venous catheterisation and blood sampling during incremental exercise test
5. Biodex muscle function tests
6. Muscle biopsies at rest and following fatiguing knee extensor exercise on Cybex.
7. 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.

Signature: ..... Date.....

Witness other than the experimenter :

Signature: ..... Date.....

CARDIOVASCULAR RISK FACTOR QUESTIONNAIRE

In order to be eligible to participate in the experiment investigating:
"Effect of 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:** _____
Date of Birth_____
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: _____

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VICTORIA UNIVERSITY OF TECHNOLOGY

Subject Information Sheet

"Skeletal muscle analysis post thoracic transplantation: control subjects"

INVESTIGATORS:

Dr. Michael J. McKenna, ph: 9688 4499 message 9688 4470
Assoc. Prof. Michael F. Carey*, ph: 9688 4298
Mr. Steve Fraser ph: 9688 4089
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.

Dear

Thankyou for volunteering to participate in this study and for taking the time to read through this information. This information sheet describes the nature of the study and the procedures involved for participants. Included are more detailed procedures and consent forms, which you need to complete and bring with you on the first day of testing. 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.

You will be required for testing on three occasions, at the Human Performance Laboratory (Room L305, Building L, V.U.T Footscray campus). A map is included showing the address and parking. Times that are suitable and the order of testing will be confirmed over the phone by Steve Fraser. Any queries in relation to the study can be directed to Steve Fraser on w:9688 4089 & h:9551 3764 or Dr. Michael McKenna on w:9688 4499, message 9688 4470.

Thankyou once again,

Yours sincerely
Steve Fraser (Ph.D. Student)

Please refrain from vigorous exercise, alcohol or tobacco for 24 hours prior to each test. Avoid food for 2 hours prior and caffeine for 4 hours prior to each exercise test.

Day 1: VO_2max test with blood sampling. You will be required to attend the Human Performance Laboratory at Footscray to perform a maximal exercise test to exhaustion 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 (detailed procedures for blood sampling are outlined in the paragraph entitled "catheterisation & blood sampling"). Please bring runners, shorts, T-shirt and a towel. This test will take approximately 1.5 hours. Shower facilities are available.

Day 2: Anthropometric measurements. At the Human Performance lab a range of non-invasive measurements will be taken including a lung function test where 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. Other tests include underwater weighing and skinfolds for % body fat, as well as leg girth, length and volume measurements. Please bring shorts T-shirt, bathers and a towel. This test will take approximately 1 hour. Shower facilities are available.

Day 3. Resting muscle biopsies . On the third visit to the Human Performance Laboratory, Footscray Campus, two resting muscle biopsies 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 (detailed procedures for muscle biopsy are outlined in the paragraph entitled "muscle biopsy procedure"). Please bring shorts. This test will take approximately 0.5 hours.

Subject participation:

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 without any adverse effects, reactions or discrimination. A summary report of your results as well as some instant feedback will be given for all tests conducted. Thank you for your co-operation.

Appendix B Individual data

Appendix B1 Subject Physical Characteristics

Table B1.1 Anthropometric data for untrained controls

				Thigh volumes		
subject	Age (yr.)	Height (cm)	Body mass (kg)	Total thigh volume (J& P) (l)	m+b volume(l)	thigh muscle C.S.A (cm^2)
1	24	186.0	85.6	5.51	4.48	211.0
2	27	188.5	80.6	5.46	4.75	198.7
3	20	172.0	65.9	4.17	3.51	169.2
4	26	181.0	80.9	5.83	4.61	193.7
5	26	182.2	84.7	7.22	5.81	234.4
6	27	184.7	82.7	6.09	4.96	204.5
7	34	181.4	79.9	6.53	5.61	216.4
8	27	190.5	87.2	5.47	4.31	186.2
n	8	8	8	8	8	8
mean	26.4	183.3	80.9	5.8	4.8	201.8
SD	3.9	5.7	6.6	0.9	0.7	19.8

	Skinfolds									
subject	Tricep (mm)	Bicep (mm)	Sub-scapular (mm)	Mid – axilla (mm)	Supra-iliac (mm)	Ab-dominal (mm)	anterior thigh (mm)	medial calf (mm)	sum of 8 (mm)	% body fat (skinfolds)
1	10.9	5.7	9.9	7.9	12.1	23.1	16.3	8.2	94.1	11.9
2	6.2	5.1	11.1	9.4	22.3	31.5	10.9	7.1	103.6	13.8
3	7.0	3.4	6.9	5.3	7.1	7.1	11.7	7.0	55.6	5.6
4	15.5	8.6	25.3	17.6	27.3	32.3	17.5	9.5	153.5	20.0
5	11.5	5.7	12.9	10.9	23.3	22.1	17.8	12.5	116.7	14.7
6	12.1	3.6	8.7	7.0	10.2	22.5	15.9	8.0	87.9	11.3
7	8.0	3.1	11.9	9.1	9.8	15.2	11.7	5.8	74.5	10.5
8	10.4	5.8	11.5	7.2	15.2	22.4	18.2	18.7	109.3	12.9
n	8	8	8	8	8	8	8	8	8	8
mean	10.2	5.1	12.3	9.3	15.9	22.0	15.0	9.6	99.4	12.6
SD	3.1	1.8	5.6	3.8	7.5	8.2	3.0	4.2	29.4	4.1

(J & P) = method of Jones and Pearson 1969, m+b = muscle + bone volume, C.S.A. = Cross sectional area

Table B1.2 Anthropometric data for endurance trained subjects

				Thigh volumes		
subject	Age (yr.)	Height (cm)	Body mass (kg)	Total thigh volume (J& P) (l)	m+b volume(l)	thigh muscle C.S.A (cm^2)
1	25	189.4	89.7	6.06	5.51	259.7
2	25	184.0	80.0	5.95	5.61	228.3
3	29	177.0	73.8	4.61	4.21	207.7
4	25	174.8	65.4	4.14	3.74	188.3
5	22	168.5	66.5	4.92	3.91	207.7
6	32	169.0	59.5	4.07	3.59	176.8
7	25	179.0	75.9	4.92	4.12	191.0
8	28	176.0	83.4	6.11	4.95	249.0
n	8	8	8	8	8	8
mean	26.4	177.2	74.3	5.1	4.5	213.6
SD	3.1	7.1	10.1	0.8	0.8	29.7

	Skinfolds									
subject	Tricep (mm)	Bicep (mm)	Sub-scapular (mm)	Mid – axilla (mm)	Supra-iliac (mm)	Ab-dominal (mm)	anterior thigh (mm)	medial calf (mm)	sum of 8 (mm)	% body fat (skinfolds)
1	10.5	6.2	14.7	12.7	17.3	17.3	18.2	9.3	106.1	21.6
2	5.2	6.3	18.5	11.3	18.8	25.3	13.0	7.4	105.7	17.3
3	18.7	7.1	9.7	15.1	17.2	27.8	27.8	15.4	138.7	29.2
4	19.3	7.5	10.2	11.0	11.5	20.3	27.6	13.3	120.7	25.7
5	8.4	4.7	7.7	7.9	16.0	14.9	22.5	9.4	91.5	20.8
6	14.8	8.1	11.0	11.2	11.8	19.8	25.8	13.0	115.4	24.0
7	11.5	5.7	12.9	10.9	23.3	22.1	17.8	12.5	116.7	14.8
8	10.4	5.8	11.5	7.2	15.2	22.4	18.2	18.7	109.3	12.9
n	8	8	8	8	8	8	8	8	8	8
mean	12.3	6.4	12.0	10.9	16.4	21.2	21.4	12.4	113.0	20.8
SD	4.9	1.1	3.4	2.5	3.8	4.2	5.4	3.6	13.7	5.5

(J & P) = method of Jones and Pearson 1969, m+b = muscle + bone volume, C.S.A. = Cross sectional area

Table B1.3 Anthropometric data for resistance trained subjects

				Thigh volumes		
subject	Age (yr.)	Height (cm)	Body mass (kg)	Total thigh volume (J& P) (l)	m+b volume(l)	thigh muscle C.S.A (cm^2)
1	20	170.5	81.6	5.57	5.05	229.3
2	37	173.5	88.1	5.78	5.05	255.8
3	37	173.5	88.1	5.78	5.05	255.8
4	20	183.0	79.3	5.41	4.81	204.9
5	27	188.5	80.6	5.46	4.75	198.7
6	26	183.6	82.9	5.57	4.99	227.2
7	18	172.9	82.4	4.87	4.12	226.3
8	39	173.0	81.4	6.19	5.50	242.0
n	8	8	8	8	8	8
mean	28.0	177.3	83.0	5.6	4.9	230.0
SD	8.6	6.7	3.3	0.4	0.4	21.1

	Skinfolds									
subject	Tricep (mm)	Bicep (mm)	Sub-scapular (mm)	Mid – axilla (mm)	Supra-iliac (mm)	Ab-dominal (mm)	anterior thigh (mm)	medial calf (mm)	sum of 8 (mm)	% body fat (skinfolds)
1	6.3	7.0	16.4	12.5	20.4	22.2	7.6	11.1	103.5	12.3
2	3.8	2.8	10.9	11.2	12.0	14.5	11.2	3.9	70.2	10.5
3	3.8	2.8	10.9	11.2	12.0	14.5	11.2	3.9	70.2	10.5
4	5.5	3.3	8.3	7.1	9.3	9.0	8.9	5.0	56.3	6.1
5	6.2	5.1	11.1	9.4	22.3	31.5	10.9	7.1	103.6	21.2
6	4.9	3.2	12.6	8.4	20.0	19.8	8.4	6.1	83.4	10.8
7	7.9	4.1	8.6	6.9	12.0	13.5	13.3	7.8	73.9	8.1
8	8.1	2.9	9.6	7.3	16.3	12.7	9.9	5.3	71.9	10.8
n	8	8	8	8	8	8	8	8	8	8
mean	5.8	3.9	11.1	9.2	15.5	17.2	10.2	6.3	79.1	11.3
SD	1.6	1.5	2.6	2.2	4.9	7.1	1.9	2.4	16.8	4.5

(J & P) = method of Jones and Pearson 1969, m+b = muscle + bone volume, C.S.A. = Cross sectional area

Table B1.4 Anthropometric data for lung transplant patients

				Thigh volumes		
subject	Age (yr.)	Height (cm)	Body mass (kg)	Total thigh volume (J& P) (l)	m+b volume(l)	thigh muscle C.S.A (cm^2)
1	43	161.0	48.0	3.40	2.27	116.8
2	53	176.0	86.5			
3	49	165.7	66.0	6.14	5.37	162.3
4	43	160.5	64.4	6.06	3.88	137.9
5	34	171.5	58.8	5.23	3.77	147.5
6	26	158.5	73.6	5.57	3.81	196.2
7	29	178.0	59.4	3.37	3.02	116.4
8	25	175.0	61.7	4.04	2.52	114.1
n	8	8	8	7	7	7
mean	37.8	168.3	64.8	4.80	3.50	141.6
SD	10.73	7.80	11.38	1.21	1.04	30.2

	Skinfolds									
subject	Tricep (mm)	Bicep (mm)	Sub-scapular (mm)	Mid – axilla (mm)	Supra-iliac (mm)	Ab-dominal (mm)	anterior thigh (mm)	medial calf (mm)	sum of 8 (mm)	% body fat (skinfolds)
1	11.7	9.8	7.4	6.4	12.3	15.9	25.3	12.3	100.9	21.8
2										
3	12.5	8.2	7.2	14.3	19.6	15.0	10.2	12.0	98.9	19.8
4	21.3	13.0	23.2	21.1	33.1	29.4	31.2	24.6	196.8	36.1
5	10.0	5.1	23.2	9.3	14.9	14.0	23.2	12.0	111.5	21.4
6	14.7	5.4	23.2	10.2	32.1	31.5	30.7	18.9	166.7	33.9
7	4.3	2.4	23.2	4.7	6.4	8.9	6.8	3.9	60.5	8.0
8	13.2	10.5	23.2	10.3	15.5	14.3	29.5	14.0	130.5	16.3
n	7	7	7	7	7	7	7	7	7	7
mean	12.5	7.8	18.7	10.9	19.1	18.4	22.4	13.9	123.7	22.5
SD	5.12	3.67	7.76	5.42	10.04	8.55	9.97	6.45	45.67	9.8

(J & P) = method of Jones and Pearson 1969, m+b = muscle + bone volume, C.S.A. = Cross sectional area

Table B1.5 Anthropometric data for age-matched controls

				Thigh volumes		
subject	Age (yr.)	Height (cm)	Body mass (kg)	Total thigh volume (J& P) (l)	m+b volume(l)	thigh muscle C.S.A (cm^2)
1	39	154.5	50.4	3.95	3.03	142.77
2	53	165.5	65.7	3.47	2.86	151.24
3	46	167.5	64.0	3.01	2.02	133.89
4	43	170.1	65.5	5.60	3.85	182.53
5	35	166.5	62.9	5.14	3.76	167.95
6	28	159.5	56.2	3.70	2.55	143.31
7	27	182.2	84.7	7.22	5.81	234.45
8	27	190.5	87.2	5.47	4.31	186.24
n	8	8	8	8	8	8
mean	37.3	169.5	67.1	4.7	3.5	167.8
SD	9.7	11.7	12.8	1.4	1.2	33.1

	Skinfolds									
subject	Tricep (mm)	Bicep (mm)	Sub-scapular (mm)	Mid – axilla (mm)	Supra-iliac (mm)	Ab-dominal (mm)	anterior thigh (mm)	medial calf (mm)	sum of 8 (mm)	% body fat (skinfolds)
1	10.5	6.2	14.7	12.7	17.3	17.3	18.2	9.3	106.1	21.6
2	5.2	6.3	18.5	11.3	18.8	25.3	13.0	7.4	105.7	17.3
3	18.7	7.1	9.7	15.1	17.2	27.8	27.8	15.4	138.7	29.2
4	19.3	7.5	10.2	11.0	11.5	20.3	27.6	13.3	120.7	25.7
5	8.4	4.7	7.7	7.9	16.0	14.9	22.5	9.4	91.5	20.8
6	14.8	8.1	11.0	11.2	11.8	19.8	25.8	13.0	115.4	24.0
7	11.5	5.7	12.9	10.9	23.3	22.1	17.8	12.5	116.7	14.8
8	10.4	5.8	11.5	7.2	15.2	22.4	18.2	18.7	109.3	12.9
n	8	8	8	8	8	8	8	8	8	8
mean	12.3	6.4	12.0	10.9	16.4	21.2	21.4	12.4	113.0	20.8
SD	4.9	1.1	3.4	2.5	3.8	4.2	5.4	3.6	13.7	5.5

(J & P) = method of Jones and Pearson 1969, m+b = muscle + bone volume, C.S.A. = Cross sectional area

Table B1.6 Measured and predicted spirometry data for untrained, endurance trained and resistance trained.

Untrained					**Endurance**				**Resistance**			
	FVC		FEV_1		FVC		FEV_1		FVC		FEV_1	
	m	p	m	p	m	p	m	p	m	p	m	p
1	5.0	6.0	4.7	4.9	6.2	6.2	4.7	5.0	5.2	5.2	4.0	4.4
2	7.1	6.1	5.4	5.0	5.4	5.9	4.3	4.8	3.9	5.0	3.0	4.1
3	5.1	5.2	4.4	4.4	6.7	5.3	5.5	4.4	3.9	5.0	3.0	4.1
4	4.4	5.7	3.6	4.7	5.2	5.3	4.0	4.4	5.8	5.9	4.9	4.9
5	5.2	5.7	4.0	4.7	4.6	5.0	3.8	4.2	7.1	5.1	5.4	4.1
6	5.8	5.9	4.1	4.8	6.2	4.8	4.6	4.0	7.3	5.8	6.5	4.8
7	5.2	5.5	4.3	4.5	4.4	5.6	3.9	4.6	5.9	5.3	5.2	4.5
8	5.0	6.2	4.4	5.0	5.2	5.3	4.8	4.4	5.2	4.9	4.3	4.0
n	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
Mean	**5.3**	**5.8**	**4.4**	**4.8**	**5.5**	**5.4**	**4.4**	**4.5**	**5.6**	**5.3**	**4.5**	**4.4**
SE	**0.28**	**0.11**	**0.19**	**0.08**	**0.28**	**0.1**	**0.20**	**0.11**	**0.45**	**0.12**	**0.42**	**0.12**

VC Vital Capacity (l), FEV_1 Forced expired volume in 1 second (l), m measured, p predicted for age and height (Crapo et al. 1981)

Table B1.7 Measured and predicted spirometry data for age-matched controls and transplant patients.

Lung Transplant Patients					**Age-matched controls**			
	FVC		FEV_1		FVC		FEV_1	
	m	p	m	p	m	p	m	p
1	3.4	3.5	2.8	2.8	4.3	3.2	2.8	2.7
2	4.8	5.0	2.1	3.8	4.2	4.1	3.8	3.4
3	3.5	3.6	1.4	2.8	4.7	3.6	3.6	2.9
4	3.4	3.5	2.4	2.8	4.2	3.8	3.3	3.1
5	4.1	4.2	3.5	3.4	5.5	3.8	4.4	3.2
6	3.6	3.8	3.2	3.1	4.6	3.6	3.8	3.1
7	5.4	5.6	3.4	4.5	5.2	5.7	4.0	4.7
8	5.3	5.6	2.6	4.4	5.0	6.2	4.4	5.0
n	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
Mean	**3.5**	**4.2**	**2.7**	**3.4**	**4.7**	**4.3**	**3.7**	**3.5**
SE	**0.3**	**0.3**	**0.3**	**0.3**	**0.2**	**0.2**	**0.2**	**0.3**

VC Vital Capacity (l), FEV_1 Forced expired volume in 1 second (l), m measured, p predicted for age and height (Crapo et al. 1981)

Appendix B2 HR and blood data for muscle fatigue test for UT, RT, ET.

K^+ variables: peak $[K^+]$, delta $[K^+]$, delta $[K^+]$.work^{-1}, fall 1 min post, fall 2 min post.

Table B2.1.1 Blood data for UT muscle fatigue test

subject		#1	#2	#3	#4	#5	#6	#7	#8	n	mean	SE
	Sample#											
HR	R	68	67	59	72	84	66	58	55	8	66	3.3
(b.min^{-1})	E1	115	98	104		103	93	120	137	8	110	5.7
	E2	128	150	99		110	116	152	141	8	128	7.8
	+1	95		70	123	82	63	68	90	7	84	7.8
	+2	92		65	105	73	58	63	69	7	75	6.5
	+5	80		54	91	72	59	62	68	7	69	4.8
	+10	80		57		78	61	69	60	7	68	4.0
	+20	80		55		74	64	64	59	7	66	3.8
	+30	79		56			61		70	7	67	5.1
bl [lac]	R	0.80	0.75	0.85	0.52	1.22	0.60	1.17	1.07	8	0.87	0.09
(mmol.l^{-1})	E1	1.47	1.39	1.60	1.67	2.06	1.40	1.85	1.33	8	1.60	0.09
	E2	2.45	2.38	3.60	3.97	4.73	1.87	3.19	1.23	8	2.93	0.41
	+1	3.43	3.73	1.69	5.48	7.61	3.89	4.59	3.03	8	4.18	0.63
	+2	3.67	4.04	6.41	6.08	4.83	4.60	5.78	3.86	8	4.91	0.37
	+5	2.17	3.60	5.35	6.77	4.40	4.69	5.51	4.09	8	4.57	0.49
	+10	1.36		5.22	6.52	3.18	3.52	4.03	2.84	7	3.81	0.63
	+20	1.37		2.88	4.07	2.20	1.74	2.48	0.97	7	2.25	0.39
	+30	1.04		1.97	2.54	1.56	1.02	1.78	0.75	7	1.52	0.24
[Hb]	R	14.6	14.0	14.9	15.4	15.7	14.7	14.4	13.5	8	14.6	0.3
(g.dl^{-1})	E1	15.0	14.1	15.3	15.9	15.7	15.2	15.0	14.0	8	15.0	0.2
	E2	15.5	14.5	15.8	16.5	16.1	15.6	15.2	14.3	8	15.4	0.3
	+1	15.4	14.6	16.1	16.7	15.9	15.6	15.3	14.7	8	15.5	0.3
	+2	15.3	14.6	16.0	16.8	15.7	15.6	15.1	14.5	8	15.4	0.3
	+5	14.9	14.3	15.9	16.4	15.7	15.4	14.7	13.8	8	15.1	0.3
	+10	14.7	14.1	15.3	16.1	15.7	15.3	14.7	13.6	8	14.9	0.3
	+20	14.4	13.6	15.0	15.3	15.3	14.9	14.4	13.5	8	14.5	0.3
	+30	14.3	14.0	14.6	15.5	15.3	14.6	14.1	13.1	8	14.4	0.3
dPV	R	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8	0.0	0.0
(%)	E1	-4.1	-1.1	-6.1	-3.5	1.7	-4.4	-4.7	-1.1	8	-2.9	0.9
	E2	-8.6	-0.2	-10.3	-9.9	-1.4	-6.3	-4.7	-4.5	8	-5.7	1.3
	+1	-7.3	-5.6	-13.1	-8.0	-2.7	-6.2	-7.2	-4.7	8	-6.9	1.1
	+2	-8.2	-5.0	-10.7	-13.3	-2.4	-7.5	-7.3	-5.4	8	-7.5	1.2
	+5	-3.9	-3.0	-15.0	-9.3	-2.6	-5.6	-3.5	-4.8	8	-5.9	1.5
	+10	-4.3	-3.5	-7.9	-6.2	0.6	-3.4	-2.5	4.1	8	-2.9	1.3
	+20	-0.5	5.5	-5.0	3.4	3.5	-1.9	0.7	2.1	8	1.0	1.2
	+30	2.0	0.6	-0.4	-1.0	2.6	1.7	3.4	7.3	8	2.0	0.9
[H$^+$]	R	36.4	35.2	39.0	37.1	38.2	37.1	38.9	38.2	8	37.5	0.5
(nmol.l^{-1})	E1	37.6	35.2	36.6	34.8	38.7	39.3	39.2	37.5	8	37.4	0.6
	E2	39.6	36.5	38.5	39.2	42.4	40.6	39.0	39.2	8	39.4	0.6
	+1	38.5	38.2	44.0	42.9	42.3	41.4	42.8	41.4	8	41.4	0.7
	+2	39.2	37.5	45.1	43.0	42.0	42.3	42.7	41.4	8	41.6	0.8
	+5	38.3	39.2	45.5	47.0	41.2	42.3	42.9	41.5	8	42.2	1.0
	+10	38.6	38.3	42.0	46.4	40.1	39.4	40.8	39.8	8	40.7	0.9
	+20	38.5	37.3	40.7	40.2	39.9	39.4	37.3	37.8	8	38.9	0.5
	+30	36.9	36.9	39.8	40.0	37.3	38.8	40.2	37.5	8	38.4	0.5
[K$^+$]	R	4.01	3.73	4.08	4.17	3.76	3.78	3.94	3.66	8	3.89	0.07
(mmol.l^{-1})	E1	4.32	4.09	4.44	4.71	3.79	3.91	4.33	4.48	8	4.26	0.11
	E2	4.79	4.57	5.01	5.81	4.39	4.16	4.86	4.91	8	4.81	0.17
	+1	4.57	4.30	4.73	4.79	3.90	4.09	4.39	4.39	8	4.39	0.11
	+2	4.14	4.24	4.35	4.19	3.84	3.93	4.07	4.06	8	4.10	0.06
	+5	3.99	3.88	4.07	3.78	3.82	3.83	3.76	3.52	8	3.83	0.06
	+10	4.02	3.89	4.13	3.87	3.82	3.93	3.94	3.79	8	3.92	0.04
	+20	3.86	3.59	4.21	3.93	3.73	3.84	3.96	3.70	8	3.85	0.07
	+30	3.84	3.73	4.09	4.18	3.64	3.80	3.79	3.68	8	3.84	0.07

Table B2.1.2 Blood data for RT muscle fatigue test

subject		#1	#2	#3	#4	#5	#6	#7	#8	n	mean	SE
	Sample#											
HR	R	79	77	99	70		65		59	6	75	5.7
($b.min^{-1}$)	E1	124	98	127	136		114		84	6	114	8.0
	E2	135	102	132	136		123		85	6	119	8.5
	+1	76	79	88	86		86		68	6	81	3.1
	+2	69	80	94	84		73		58	6	76	5.1
	+5	70	80	84	77		62		52	6	71	4.9
	+10	65	74	78	82		72		50	6	70	4.7
	+20	70	71	76	73		72		55	6	70	3.0
	+30	70	73	76	75		74		55	6	71	3.2
bl [lac]	R	1.98	1.24	1.50	0.88	0.70	0.29		0.66	7	1.04	0.22
($mmol.l^{-1}$)	E1	2.43	1.44	2.59	2.03	1.87	2.18		1.29	7	1.97	0.18
	E2	3.42	1.76	3.38	3.87	3.38	3.01		1.85	7	2.95	0.31
	+1	4.74	2.59		5.31	4.40	4.81		2.47	6	4.05	0.50
	+2	5.76	2.80		6.76	4.04	4.81		2.82	6	4.50	0.65
	+5	6.09	3.97		7.44	5.05	3.48		1.97	6	4.67	0.80
	+10	4.55	3.81	4.02	6.22	4.58	2.71		1.78	7	3.95	0.54
	+20	2.74	2.88	2.40	3.10	2.59	1.54		1.27	7	2.36	0.26
	+30	1.98	2.00	1.70	1.72	1.70	1.51		1.00	7	1.66	0.13
[Hb]	R	16.1	14.3	15.1	15.2	13.2	15.4		14.2	7	14.8	0.4
($g.dl^{-1}$)	E1	16.0	14.3	15.7	16.0	13.2	15.5		14.2	7	15.0	0.4
	E2	16.3	14.5	16.1	16.0	14.0	15.8		14.7	7	15.3	0.4
	+1	16.4	14.7		16.1	14.1	15.9		15.1	7	15.4	0.4
	+2	16.3	14.6		16.2	14.0	15.6		15.0	7	15.3	0.4
	+5	15.8	14.7	15.7	16.0	13.6	15.3		14.5	7	15.1	0.3
	+10	15.7	14.4	15.4	15.5	13.4	15.3		14.4	7	14.9	0.3
	+20	15.5	14.0	15.1	15.1	13.0	15.1		14.2	7	14.6	0.3
	+30	15.4	13.7	14.8	15.4	13.1	15.5		14.3	7	14.6	0.4
dPV	R	0.0	0.0	0.0	0.0	0.0	0.0		0.0	7	0.0	0.0
(%)	E1	-2.4	3.5	-2.3	-6.9	-4.4	2.3		-2.4	7	-1.8	1.4
	E2	1.2	-1.9	-9.6	-5.4	-9.8	2.7		-4.5	7	-3.9	1.8
	+1	-1.7	-2.4		-6.4	-10.7	-0.8		-9.5	7	-5.3	1.7
	+2	-4.2	-2.6		-7.1	-12.5	-0.4		-8.1	7	-5.8	1.8
	+5	-1.5	-3.0	-4.3	-6.6	-5.7	8.8		-6.0	7	-2.6	2.0
	+10	1.3	2.2	-6.1	-2.1	-3.2	7.6		-4.2	7	-0.7	1.8
	+20	3.5	5.1	-3.9	4.4	0.0	1.0		0.0	7	1.4	1.2
	+30	5.9	8.5	2.7	2.8	-2.3	0.1		-4.6	7	1.9	1.7
$[H^+]$	R	36.9	38.3	38.1	38.5	36.2	36.3		38.1	7	37.5	0.4
($nmol.l^{-1}$)	E1	31.7	40.5	38.2	38.1	35.3	33.0		37.9	7	36.4	1.2
	E2	33.2	42.4	38.5	38.8	39.9	35.6		39.9	7	38.3	1.1
	+1	37.2	40.8		44.2	40.0	41.5		40.4	6	40.7	0.9
	+2	38.2	42.8		46.3	41.1	42.1		40.8	6	41.9	1.1
	+5	40.1	44.0	40.2	47.6	44.2	40.7		39.7	7	42.4	1.1
	+10	38.2	43.2	39.1	44.2	42.0	38.3		38.2	7	40.4	1.0
	+20	34.1	40.5	38.2	42.0	38.9	36.4		37.8	7	38.3	1.0
	+30	32.8	39.4	38.1	37.8	36.9	36.4		35.3	7	36.7	0.8
$[K^+]$	R	3.78	3.98	3.77	4.56	4.08	3.75		3.86	7	3.97	0.11
($mmol.l^{-1}$)	E1	3.85	4.08	4.15	4.77	4.48	3.93		4.02	7	4.18	0.12
	E2	4.18	4.27	4.27	5.16	5.31	4.41		4.41	7	4.57	0.17
	+1	4.10	4.14		4.99	4.56	3.96		4.28	6	4.34	0.15
	+2	3.93	4.22		4.63	4.11	3.60		4.11	6	4.10	0.14
	+5	3.53	4.19	3.61	4.32	3.78	3.39		3.85	7	3.81	0.13
	+10	3.68	4.12	3.81	4.30	3.92	3.62		3.95	7	3.91	0.09
	+20	3.80	3.96	3.78	4.31	3.95	3.65		3.90	7	3.91	0.08
	+30	3.85	3.99	3.72	4.41	4.47	3.74		3.91	7	4.01	0.12

Table B2.1.3 Blood data for ET muscle fatigue test

subject		#1	#2	#3	#4	#5	#6	#7	#8	n	mean	SE
	Sample#											
HR	**R**	74	60	54	46	72	75	67	70	8	65	3.7
($b.min^{-1}$)	**E1**	129	126	105	104	116	148	145	115	8	124	5.9
	E2	150	127		111	136	170	157	123	8	139	7.9
	+1	101	69		59	115		99	69	8	85	9.2
	+2	82	60		51			53	72	8	64	5.9
	+5	72	63		58				68	8	65	3.0
	+10	72	60		52	69		70	72	8	66	3.3
	+20	72	55		53			64	68	8	62	3.7
	+30	70	60		62		75	66	68	8	67	2.2
bl [lac]	**R**	1.39		0.83	1.40	0.92	1.07	1.06	1.10	7	1.11	0.08
($mmol.l^{-1}$)	**E1**	1.94		1.51	1.91	1.98	1.58	1.24	1.13	7	1.61	0.13
	E2	2.86		1.77	2.24	2.92	2.89	1.88	2.88	7	2.49	0.19
	+1	3.84		2.47	3.75	3.65	3.87	1.94	3.36	7	3.27	0.29
	+2	3.62		2.71	4.09	3.40	4.54	2.66	2.55	7	3.37	0.29
	+5	3.19		2.35	3.35	3.96	4.44	1.54	2.72	7	3.08	0.37
	+10	2.45		1.54		3.11	3.76	1.20	1.77	7	2.30	0.40
	+20	1.74		1.49		1.84	2.24	0.93	1.17	7	1.57	0.19
	+30	1.78		0.69	1.37	1.57	1.61	0.26	0.89	7	1.17	0.21
[Hb]	**R**	14.0	14.6	15.5	15.1	16.1	14.3	13.6	15.5	8	14.8	0.3
($g.dl^{-1}$)	**E1**	14.6	14.4	15.7	15.5	16.2	14.6	14.0	15.7	8	15.1	0.3
	E2	14.6	14.9	15.9	16.1	16.7	14.9	14.2	15.9	8	15.4	0.3
	+1	14.5	15.2	15.8	16.0	16.6	14.7	14.4	16.0	8	15.4	0.3
	+2	14.1	14.8	15.9	16.0	16.5	15.0	14.2	16.0	8	15.3	0.3
	+5	14.2	14.8	14.8	15.5	15.8	14.3	13.6	15.5	8	14.8	0.3
	+10	14.1	14.0	15.3	15.6	15.4	14.4	13.7	15.5	8	14.7	0.3
	+20	13.9	13.9	15.1	15.3	15.9	13.9	13.5	15.4	8	14.6	0.3
	+30	13.6	14.2	15.2	15.0	15.6	14.2	13.7	15.4	8	14.6	0.3
dPV	**R**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8	0.0	0.0
(%)	**E1**	-7.3	3.2	-3.0	-5.8	-0.1	-1.8	-7.4	4.9	8	-2.2	1.6
	E2	-5.5	-2.3	-2.1	-11.4	-0.6	-5.9	-7.4	-8.0	8	-5.4	1.3
	+1	-6.7	-4.3	-4.2	-11.3	-5.9	-5.7	-7.9	-9.9	8	-7.0	0.9
	+2	-3.2	-2.5	-7.6	-10.3	-3.9	-6.5	-7.2	-8.4	8	-6.2	1.0
	+5	-2.8	-0.9	4.6	-9.1	2.7	2.3	-3.4	-2.9	8	-1.2	1.5
	+10	-3.1	4.1	1.5	-7.6	4.6	-0.5	-1.5	3.8	8	0.2	1.5
	+20	-0.2	5.6	6.9	-5.3	1.0	4.6	1.5	-5.1	8	1.1	1.6
	+30	3.6	5.7	1.2	-3.4	2.8	3.5	-2.4	-5.7	8	0.7	1.4
[H^+]	**R**	36.9	38.4	36.9	37.1	38.1	36.3	39.1	38.4	8	37.6	0.3
($nmol.l^{-1}$)	**E1**	36.3	39.2	36.8	37.5	36.2	35.0	36.7	37.2	8	36.9	0.4
	E2	37.7	41.3	37.9	39.0	36.8	36.0	38.1	39.9	8	38.3	0.6
	+1	39.0	42.5	38.2	40.7	42.8	40.3	38.4	38.9	8	40.1	0.6
	+2	39.7	42.3	38.2	40.5	44.7	41.8	38.9	40.1	8	40.8	0.7
	+5	39.3	40.9	37.3	40.6	44.3	41.4	39.4	38.4	8	40.2	0.8
	+10	37.7	40.5	37.1	39.2	40.6	39.7	38.5	38.5	8	39.0	0.4
	+20	38.1	38.0	35.7	37.7	39.4	37.7	37.5	31.8	8	37.0	0.8
	+30	36.8	37.2	35.9	37.1	37.6	36.7	38.7	36.5	8	37.0	0.3
[K^+]	**R**	3.85	3.83	3.54	3.78	3.79	3.68	3.77	3.80	8	3.75	0.04
($mmol.l^{-1}$)	E1	4.24	3.85	3.97	4.27	4.12	4.03	4.57	4.06	8	4.14	0.08
	E2	4.80	4.43	4.31	4.83	4.61	4.47	5.05	4.32	8	4.60	0.09
	+1	4.42	4.31	4.06	4.35	4.26	4.17	4.69	3.99	8	4.28	0.08
	+2	3.83	4.01	3.91	4.01	3.86	3.89	4.30	3.74	8	3.94	0.06
	+5	3.79	3.72	3.50	3.67	3.54	3.50	3.95	3.57	8	3.65	0.06
	+10	3.89	3.72	3.70	3.91	3.76	3.70	4.06	3.78	8	3.81	0.04
	+20	3.90	3.62	4.03	3.76	3.82	3.74	3.96	3.66	8	3.81	0.05
	+30	3.71	3.74	3.98	3.78	3.91	3.86	3.85	3.79	8	3.83	0.03

Table B2.1.4 Plasma [K^+] response during the muscle fatigue test for UT, RT, and ET

subject						
UT	peak [K^+] ($mmol.l^{-1}$)	total work (J)	Δ [K^+] ($mmol.l^{-1}$)	Δ [K^+] .$work^{-1}$ ($nmol.l^{-1}$).J^{-1}	fall in [K^+] 1 min post ($mmol.l^{-1}$)	fall in [K^+] 2 min post ($mmol.l^{-1}$)
#1	4.79	11174	0.78	69.80	0.22	0.65
#2	4.57	11775	0.84	70.91	0.27	0.33
#3	5.01	9960	0.93	93.37	0.28	0.66
#4	5.81	10236	1.64	159.72	1.02	1.62
#5	4.39	11358	0.63	55.47	0.49	0.55
#6	4.16	9568	0.38	39.71	0.07	0.23
#7	4.86	9954	0.92	92.43	0.47	0.79
#8	4.91	11920	1.25	104.45	0.52	0.85
n	**8**	**8**	**8**	**8**	**8**	**8**
mean	**4.81**	**10743**	**0.92**	**86**	**0.42**	**0.71**
SD	**0.49**	**917**	**0.38**	**37**	**0.29**	**0.42**
SEM	**0.17**	**324**	**0.13**	**13**	**0.10**	**0.15**

RT	peak [K^+] ($mmol.l^{-1}$)	total work (J)	Δ [K^+] ($mmol.l^{-1}$)	Δ [K^+] .$work^{-1}$ ($nmol.l^{-1}$).J^{-1}	fall in [K^+] 1 min post ($mmol.l^{-1}$)	fall in [K^+] 2 min post ($mmol.l^{-1}$)
#1	4.18	8423	0.41	48.08	0.08	0.13
#2	4.27	8730	0.29	32.65	0.13	0.02
#3	4.27	11892	0.50	42.05		
#4	5.16	10530	0.60	56.98	0.17	0.27
#5	5.31	9854	1.24	125.33	0.76	0.60
#6	4.41	13643	0.66	48.01	0.45	0.40
#7		7593				
#8	4.41	8443	0.55	65.14	0.13	0.15
n	**7**	**8**	**7**	7	**6**	**6**
mean	**4.57**	**9888**	**0.60**	**60**	**0.28**	**0.26**
SD	**0.46**	**2048**	**0.30**	**31**	**0.27**	**0.21**
SEM	**0.17**	**724**	**0.12**	**12**	**0.11**	**0.09**

ET	peak [K^+] ($mmol.l^{-1}$)	total work (J)	Δ [K^+] ($mmol.l^{-1}$)	Δ [K^+] .$work^{-1}$ ($nmol.l^{-1}$).J^{-1}	fall in [K^+] 1 min post ($mmol.l^{-1}$)	fall in [K^+] 2 min post ($mmol.l^{-1}$)
#1	4.8	12164	0.95	78.10	0.38	0.49
#2	4.43	10824	0.60	55.43	0.12	0.21
#3	4.31	9957	0.77	77.33	0.25	0.20
#4	4.83	10672	1.05	98.39	0.48	0.41
#5	4.61	11304	0.83	72.98	0.35	0.38
#6	4.465	10822	0.79	72.54	0.30	0.29
#7	5.05	10527	1.29	122.07	0.36	0.38
#8	4.32	11793	0.52	44.09	0.33	0.29
n	**8**	**8**	**8**	**8**	**8**	**8**
mean	**4.60**	**11008**	**0.85**	**78**	**0.32**	**0.33**
SD	**0.27**	**713**	**0.25**	**24**	**0.10**	**0.10**
SEM	**0.09**	**252**	**0.09**	**9**	**0.04**	**0.04**

Table B2.2.1 Blood data for UT for incremental exercise test (blood [lactate] and [Hb])

UT VO2 peak test	Work-load		subject										
[bl lac] ($mmol.l^{-1}$)	Watts	Sample#	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	0.48	0.52	0.82	0.87	0.52	0.51	0.75	0.32	8	0.60	0.07
	25	E1	1.00	0.56	0.72	1.37	0.60	0.48	0.69	0.50	8	0.74	0.11
	50	E2	0.96	0.66	0.98	1.19	0.70	0.99	0.79	0.82	8	0.89	0.06
	75	E3	1.44	1.24	1.03	1.43	0.71	1.54	1.03	0.76	8	1.15	0.11
	100	E4	1.37	1.06	1.46	1.87		2.14	1.16	0.87	7	1.42	0.17
	125	E5	1.43	0.76	1.91	1.94	1.03	2.97	1.16	1.09	8	1.54	0.25
	150	E6	1.68	1.21	2.48	2.72	0.88	3.51	1.50	1.03	8	1.88	0.33
	175	E7	2.18	2.10	3.51	3.20	1.46	3.80	2.15	1.71	8	2.51	0.31
	200	E8	2.59	2.32	3.76	4.21	1.94	6.24	2.65	2.14	8	3.23	0.51
	225	E9	3.23	3.12	5.51	5.22	2.47	8.16	3.58	2.68	8	4.25	0.68
	250	E10	4.16	4.10	6.60	6.43	3.61	9.68	4.86	3.86	8	5.41	0.73
	275	E11	5.62	5.08	9.64	7.97	5.41	9.75	5.58	3.93	8	6.62	0.78
	300	E12	9.59	5.25	12.14	10.64	5.89	12.55	8.43	6.59	8	8.89	0.99
	325	E13	8.72	5.39			7.68		10.65	8.59	6	8.21	0.85
	350	E14								10.62	4	10.62	
	375	E15											
	400	E16											
	425	E17											
	450	E18											
	475	E19											
	500	E20											
		+1	11.07	10.64	14.14	10.57	10.02	18.74	12.35	13.23	8	12.59	1.01
		+2	11.24	10.67	13.30	11.48	10.49	16.00	15.31	14.09	8	12.82	0.76
		+5	11.32	10.53	14.39	12.44	10.17	20.31	13.49	14.05	8	13.34	1.14
		+10	9.53	9.77	9.42	12.80	8.79	15.55	12.42	13.95	8	11.53	0.88
		+20	5.45	7.10	9.33	11.11	4.92	5.95	8.39	11.21	8	7.93	0.87
		+30	2.64	3.62	5.51	6.50	2.62	4.58	5.94	6.96	8	4.80	0.60
[Hb]	Work-load		subject										
($g.dl^{-1}$)	Watts	Sample#	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	14.7	14.3	15.7	15.9	15.2	14.8	14.2	14.4	8	14.9	0.2
	25	E1	15.1	14.4	15.8	16.3	15.1	15.1	14.6	14.2	8	15.1	0.3
	50	E2	15.1	14.8	16.0	16.5	15.3	15.0	14.4	14.7	8	15.2	0.3
	75	E3	15.3	14.7	16.0	16.7	15.4	15.1	14.7	14.6	8	15.3	0.3
	100	E4	15.1	14.8	16.1	17.1	15.5	15.0	14.8	15.0	8	15.4	0.3
	125	E5	15.4	14.9	16.3	17.0	15.6	15.3	14.8	14.9	8	15.5	0.3
	150	E6	15.5	14.7	16.6	17.0	15.7	15.4	14.9	15.0	8	15.6	0.3
	175	E7	15.4	15.0	16.6	15.5	15.5	15.5	15.2	14.9	8	15.4	0.2
	200	E8	15.7	15.2	16.7	17.3	15.7	15.7	15.2	15.2	8	15.8	0.3
	225	E9	15.5	15.1	17.1	17.4	16.0	15.8	15.3		7	16.0	0.3
	250	E10	15.6	15.1	17.3		16.1	16.0	15.4	15.6	7	15.9	0.3
	275	E11	16.0	15.3	17.4		16.3	16.2	15.4	15.7	7	16.0	0.3
	300	E12	16.3		17.9		16.5	16.5	15.7		5	16.6	0.4
	325	E13	16.3	15.4					15.8	16.3	4	15.9	0.2
	350	E14								16.6	2	16.6	
	375	E15											
	400	E16											
	425	E17											
	450	E18											
	475	E19											
	500	E20											
		+1	16.1	15.7	16.7	16.1	17.0	16.4	16.1	16.8	8	16.4	0.2
		+2	15.9	15.4	15.7	16.9	16.9	16.5	16.0	16.9	8	16.3	0.2
		+5	15.7	15.3	16.6	16.6	16.5	16.3	15.8	16.2	8	16.1	0.2
		+10	15.2		16.3	16.3	16.2	16.0	15.7	16.1	8	16.0	0.1
		+20	15.0	14.1	15.9	15.9	15.7	15.3	14.9	15.6	8	15.3	0.2
		+30	14.8	13.7	15.5	15.5	15.7	14.8	15.0	15.3	8	15.0	0.2

Table B2.2.1 Blood data for UT for incremental exercise test (Δ plasma volume and $[H^+]$)

dPV (%)	Work-load Watts	Sample#	subject #1	#2	#3	#4	#5	#6	#7	#8	n	mean	SE
	0	R	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8	0.0	0.0
	25	E1	-7.7	2.9	2.4	-1.2	0.2	-5.3	-2.3	1.0	8	-1.2	1.3
	50	E2	-6.4	2.0	-2.4	-0.8	-0.6	-3.1	-0.9	-2.1	8	-1.8	0.9
	75	E3	-8.2	3.0	0.2	-2.0	-1.4	-6.2	-4.4	-0.3	8	-2.4	1.3
	100	E4	-8.9	0.5	-1.3	-4.1	-3.6	-5.6	-6.2	-3.1	8	-4.0	1.0
	125	E5	-9.5	-0.9	-4.2	-6.8	-3.1	-6.7	-4.9	-4.9	8	-5.1	0.9
	150	E6	-10.0	0.4	-6.9	-4.9	-3.4	-8.0	-6.7	-3.5	8	-5.4	1.1
	175	E7	-7.4	-1.0	-6.0	5.2	-3.3	-9.4	-7.1	-5.1	8	-4.3	1.6
	200	E8	-9.9	-4.4	-8.5	-6.0	-3.8	-9.7	-7.1	-9.0	8	-7.3	0.8
	225	E9	-9.6	-3.5	-11.7	-8.0	-6.9	-11.8	-11.1		7	-9.0	1.2
	250	E10	-12.1	-4.6	-12.0		-11.0	-12.7	-9.7	-10.1	7	-10.3	1.0
	275	E11	-11.7	-4.2	-14.3		-10.7	-14.2	-11.3	-14.0	7	-11.5	1.3
	300	E12	-13.6		-15.6		-11.5	-16.2	-13.6		5	-14.1	0.8
	325	E13	-15.5	-6.3			-12.8			-4.5	4	-9.8	2.6
	350	E14								-19.1	1	-19.1	
	375	E15											
	400	E16											
	425	E17											
	450	E18											
	475	E19											
	500	E20											
		+1	-14.0	-11.8	-11.9	-1.7	-13.3	-16.9	-17.9	-21.7	8	-13.7	2.1
		+2	-12.1	-7.3	-0.1	-10.1	-15.5	-17.2	-17.9	-24.9	8	-13.1	2.7
		+5	-10.6	-6.4	-7.4	-7.4	-14.4	-18.4	-16.6	-20.4	8	-12.7	1.9
		+10	-6.0		-1.6	-1.6	-10.3	-16.4	-18.8	-19.5	8	-10.6	2.9
		+20	-7.6	1.9	2.8	2.8	-6.5	-7.9	-12.1	-13.6	8	-5.0	2.4
		+30	-1.4	11.0	0.9	0.9	-3.8	-3.6	-12.0	-14.3	8	-2.8	2.8

$[H^+]$ ($nmol.l^{-1}$)	Work-load Watts	Sample#	subject #1	#2	#3	#4	#5	#6	#7	#8	n	mean	SE
	0	R	35.8	38.1	39.9	38.0	37.7	36.4	38.1	37.3	8	37.6	0.4
	25	E1	36.7	38.4	39.6	36.3	38.2	38.1	38.6	37.6	8	37.9	0.4
	50	E2	38.0	38.3	40.0	35.8	39.4	39.2	39.7	38.0	8	38.6	0.5
	75	E3	38.2	39.5	41.0	36.4	40.2	38.6	37.5	38.5	8	38.7	0.5
	100	E4	38.9	41.1	42.5	38.2	40.9	39.0	39.3	39.6	8	39.9	0.5
	125	E5	39.6	42.6	43.5	39.4	41.3	39.1	39.3	39.7	8	40.5	0.6
	150	E6	40.5	43.7	45.5	39.9	41.9	39.4	40.0	40.6	8	41.4	0.8
	175	E7	41.5	45.2	46.5	39.9	42.2	39.2	40.0	41.2	8	42.0	0.9
	200	E8	42.1	46.6	50.4	40.6	43.3	39.9	40.1	42.0	8	43.1	1.3
	225	E9	44.7	48.3	53.2	40.8	43.7	39.9	40.6	43.1	8	44.3	1.6
	250	E10	46.9	49.7		41.7	44.0	40.3	41.7	43.0	7	43.9	1.3
	275	E11	47.5	54.0		41.4	45.6	40.7	42.6	44.0	7	45.1	1.7
	300	E12		57.9		42.2	45.8	42.2	43.7	45.0	7	46.1	2.4
	325	E13	51.5			43.6		45.6	44.9	45.8	5	46.3	1.4
	350	E14							50.6	50.6	3	50.6	0.0
	375	E15											
	400	E16											
	425	E17											
	450	E18											
	475	E19											
	500	E20											
		+1	56.5	69.6	56.9	52.4	54.2	54.1	63.9	57.2	8	58.1	2.0
		+2	58.6	71.4	62.9	53.4	56.7	55.3	67.6	59.7	8	60.7	2.2
		+5	61.2	76.1	64.3	54.3	60.3	61.7	75.8	62.8	8	64.6	2.7
		+10	60.8	70.6	63.0	51.5	58.1	60.6	73.6	60.4	8	62.3	2.5
		+20	48.8	55.0	53.8	43.0	48.7	50.4	65.3	50.1	8	51.9	2.3
		+30	41.8	45.9	47.2	39.5	42.6	43.8	51.1	43.4	8	44.4	1.3

Table B2.2.1 Blood data for UT for incremental exercise test ($[K^+]$)

$[K^+]$	Work-load		subject										
(mmol.l^{-1})	Watts	Sample#	#1	#2	#3	#4	#5	#6	#7	#8	n	mean	SE
	0	R	4.03	4.18	3.80	3.88	3.66	3.94	4.04	4.09	8	3.95	0.06
	25	E1	4.24	4.13	3.94	5.00	3.63	3.97	4.23	4.29	8	4.18	0.14
	50	E2	4.36	4.39	4.10	5.02	3.84	4.05	4.21	4.55	8	4.31	0.13
	75	E3	4.52	4.47	4.25	5.10	3.96	4.13	4.37	4.59	8	4.42	0.12
	100	E4	4.47	4.61	4.48	5.23	4.05	4.23	4.48	4.64	8	4.52	0.12
	125	E5	4.44	4.63	4.66	5.39	4.16	4.36	4.49	4.72	8	4.61	0.13
	150	E6	4.58	4.79	4.86	5.42	4.27	4.52	4.62	5.42	8	4.81	0.15
	175	E7	4.65	4.86	4.94	5.77	4.32	4.61	4.74	4.77	8	4.83	0.15
	200	E8	4.63	4.47	5.14	5.68	4.46	4.90	4.94	4.97	8	4.90	0.14
	225	E9	4.53	5.08	5.50	5.96	4.68	4.99	5.12	5.57	8	5.18	0.17
	250	E10	4.72	5.26	5.75		5.02	5.12	5.36	5.64	7	5.26	0.14
	275	E11	4.99	5.46	6.05		5.36	5.46	5.65	5.63	7	5.51	0.12
	300	E12	5.29		6.64		5.29	5.75	5.86	6.22	6	5.84	0.22
	325	E13	5.72	6.07			5.65		6.28	7.39	6	6.22	0.31
	350	E14								7.02	1	7.02	
	375	E15											
	400	E16											
	425	E17											
	450	E18											
	475	E19											
	500	E20											
		+1	4.85	5.67	5.92	5.82	5.43	5.12	5.52	6.60	8	5.61	0.19
		+2	3.98	5.18	4.80	4.69	4.63	4.28	4.70	5.74	8	4.75	0.19
		+5	3.58	4.46	4.03	4.08	3.86	3.80	4.09	4.74	8	4.08	0.13
		+10	3.60	4.72	4.16	4.13	3.80	3.97	3.96	4.56	8	4.11	0.13
		+20	3.72	4.55	4.36	4.27	3.64	3.82	3.92	4.58	8	4.11	0.13
		+30	3.71	4.64	4.26	4.17	3.68	3.78	4.08	4.31	8	4.08	0.12

Table B2.2.2 Blood data for RT for incremental exercise test (blood [lactate] and [Hb])

RT VO2 peak test	Work-load		subject										
[bl lac] (mmol.l^{-1})	Watts	Sample#	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	0.90	1.07	0.82	0.46	0.67	1.09	0.80	1.12	8	0.87	0.08
	25	E1	0.66	1.02	1.20	0.37	0.76	1.37	1.09	1.19	8	0.96	0.12
	50	E2	0.99	1.07	1.19	0.65	1.24	1.76	1.14	1.33	8	1.17	0.11
	75	E3	0.95	1.29	1.45	1.25	1.47	1.57	1.51	1.26	8	1.34	0.07
	100	E4	1.39	1.77	1.80	1.49	1.50	1.81	1.57	1.60	8	1.62	0.06
	125	E5	1.42	2.28	2.12	2.43	2.04	2.20	1.17	0.49	8	1.77	0.24
	150	E6	1.59	2.82	2.58	3.09	2.48	1.58	1.70	2.00	8	2.23	0.21
	175	E7	2.30	3.59	3.37	3.79	3.22	2.52	1.67	2.65	8	2.89	0.26
	200	E8	2.47	4.08	4.02	4.60	4.29	2.84	1.77	3.77	8	3.48	0.35
	225	E9	2.77	5.74	5.00	5.79	5.72	3.33	1.70	3.99	8	4.25	0.55
	250	E10	3.78	7.08	8.10	6.33	8.02	3.83	1.93	4.50	8	5.45	0.80
	275	E11	4.96	8.43	10.07	7.36		4.81	2.34	4.23	7	6.03	1.02
	300	E12	6.35		12.56	9.64		6.26	2.78	8.11	6	7.62	1.36
	325	E13	7.76			11.43		6.93	2.25		4	7.09	1.89
	350	E14				13.04		9.00	4.44	9.53	4	9.00	1.77
	375	E15						12.40	5.04		2	8.72	3.68
	400	E16							7.03		1	7.03	
	425	E17											
	450	E18											
	475	E19											
	500	E20											
		+1	9.92	10.37	15.96	14.80	9.70	13.98	7.34	14.92	8	12.12	1.12
		+2	11.36	10.62	15.66	14.76	9.60	15.81	15.81	15.60	8	13.65	0.94
		+5	20.04	10.76	15.20	15.62	10.01	11.39	10.97	14.59	8	13.57	1.21
		+10	10.86	9.71	20.35	15.65	8.52	10.17	5.41		7	11.52	1.87
		+20	6.68	7.43	10.02	12.06	6.56	6.46	3.33		7	7.50	1.06
		+30	4.49	3.88	6.24	6.79	4.58	3.87	1.83		7	4.52	0.62
[Hb]	Work-load		subject										
(g.dl^{-1})	Watts	Sample#	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	15.1	14.3	15.3	15.5	13.6	16.1	15.1	14.6	8	14.9	0.3
	25	E1	15.5	14.4	15.7	15.7	13.9	16.1	15.3	14.9	8	15.2	0.3
	50	E2	15.5	14.7	15.8	15.9	14.0	16.6	15.3	15.2	8	15.4	0.3
	75	E3	15.8	14.6	15.9	16.0	13.9	16.4	15.1	14.8	8	15.3	0.3
	100	E4	15.8	14.8	16.0	16.1	14.2	16.5	15.3	15.2	8	15.5	0.3
	125	E5	16.2	15.0	16.2	16.2	14.2	16.4	15.3	15.0	8	15.6	0.3
	150	E6	16.0	15.2	16.2	16.4	14.4	16.8	15.5	15.1	8	15.7	0.3
	175	E7	16.3	15.3	16.2	16.4	14.6	16.3	15.4	15.4	8	15.7	0.2
	200	E8	16.0	15.6	16.2	16.4	14.7	16.5	15.4	15.3	8	15.8	0.2
	225	E9	16.3	16.1	16.1	16.5	14.7	16.6	15.4	15.6	8	15.9	0.2
	250	E10	16.6	16.0	16.4	16.7		16.7	15.2		6	16.3	0.2
	275	E11	16.8	16.3	16.6	16.8		16.5	15.5		6	16.4	0.2
	300	E12	16.9		16.6	17.0		16.8	15.7		5	16.6	0.2
	325	E13	17.1		16.8	17.1		16.8	15.5		5	16.7	0.3
	350	E14	17.2			17.4		17.1			3	17.2	0.1
	375	E15						16.7			1	16.7	
	400	E16											
	425	E17											
	450	E18											
	475	E19											
	500	E20											
		+1	17.5	16.3	17.0	17.3	15.3				5	16.7	0.4
		+2	17.2	16.3	16.9	17.3			15.8	16.3	6	16.6	0.2
		+5	16.9	16.0	16.8	17.0		17.1	15.6	15.7	7	16.4	0.2
		+10	16.5	15.5	16.4	16.5		16.8	15.6	15.5	7	16.1	0.2
		+20	15.7	15.1	15.8	15.8	14.0	16.3	14.7	15.0	8	15.3	0.3
		+30	15.5	14.6	15.3	15.7	13.6	12.8	14.1	13.8	8	14.4	0.4

Table B2.2.2 Blood data for RT for incremental exercise test (Δ plasma volume and $[H^+]$)

dPV	Work-load		subject										
(%)	Watts	Sample #	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8	0.0	0.0
	25	E1	-5.3	-2.7	1.9	8.2	-6.2	-1.8	-5.2	-3.3	8	-1.8	1.7
	50	E2	-5.6	-2.7	-4.2	-7.0	-4.7	-4.1	-4.8	-0.6	8	-4.2	0.7
	75	E3	-6.1	-3.1	-4.3	0.9		-2.3	-3.7	-0.8	7	-2.8	0.9
	100	E4	-5.3	-4.7	-6.4	-2.2	-5.9	-5.6	-6.5	-4.8	8	-5.2	0.5
	125	E5	-9.1	-6.3	-8.0	-8.1	-9.2	-4.5	-2.0	-0.3	8	-5.9	1.2
	150	E6	-9.4	-7.8	-8.6	-3.1	-9.6	-7.5	-3.5	-2.8	8	-6.5	1.0
	175	E7	-8.8	-8.1	-8.5	-3.9	-11.5	-0.7	-3.7	-2.1	8	-5.9	1.3
	200	E8	-9.0	-12.4	-10.1	-8.0	-11.6	-5.3	-2.8	-3.7	8	-7.9	1.3
	225	E9	-11.7	-15.0	-8.1	-13.1	-14.0	-4.6	-9.8	-7.9	8	-10.5	1.3
	250	E10	-11.4	-15.8	-10.4	-10.3		-5.2	-4.9		6	-9.7	1.7
	275	E11	-13.0	-15.8	-12.9	-12.0		-3.9	-5.2		6	-10.5	1.9
	300	E12	-14.2		-12.7	-13.2		-6.9	-9.9		5	-11.4	1.3
	325	E13	-16.3		-13.9	-18.6		-3.9	-5.8		5	-11.7	2.9
	350	E14	-16.2			-13.3		-9.1			3	-12.9	2.1
	375	E15						-9.7			1	-9.7	
	400	E16											
	425	E17											
	450	E18											
	475	E19											
	500	E20											
		+1	-19.7	-17.3	-16.5	-10.9	-17.1				5	-16.3	1.5
		+2	-19.0	-18.5	-16.8	-8.5			-9.5	-13.1	6	-14.2	1.9
		+5	-17.4	-17.0	-15.0	-15.0		-10.3	-11.5	-9.4	7	-13.7	1.2
		+10	-15.2	-10.7	-11.3	-6.6		-6.4	-5.7	-5.7	7	-8.8	1.4
		+20	-4.9	-8.1	-6.5	0.1	-4.1	2.1	-13.7	0.5	8	-4.3	1.8
		+30	-2.8	-1.5	0.6	0.4	-0.7		7.1	11.0	7	2.0	1.9
$[H^+]$	Work-load		subject										
($nmol.l^{-1}$)	Watts	Sample #	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	34.0	36.6	38.7	34.5	35.7	38.1	36.5	35.8	8	36.2	0.6
	25	E1	37.8	37.3	34.2	34.5	36.1	36.6	37.8	36.4	8	36.3	0.5
	50	E2	39.6	38.0	33.3	35.5	36.1	39.1	38.6	37.2	8	37.2	0.7
	75	E3	40.8	38.0	35.8	36.3	35.6	39.9	39.0	37.9	8	37.9	0.7
	100	E4	41.6	39.9	39.2	38.3	37.2	40.4	38.5	39.4	8	39.3	0.5
	125	E5	42.5	39.9	41.4	39.1	39.4	39.8	39.8	40.4	8	40.3	0.4
	150	E6	42.6	41.1	42.9	38.9	38.5	40.7	39.9	40.8	8	40.7	0.5
	175	E7	44.8	41.9	43.2	40.4	38.9	41.2	40.9	41.8	8	41.6	0.6
	200	E8	45.7	43.0	45.2	42.8	39.9	41.7	39.3	42.6	8	42.5	0.8
	225	E9	47.6	44.2	46.8	44.4	39.5	41.8	39.6	43.5	8	43.4	1.1
	250	E10	49.2	44.0	48.1		38.5	42.1	36.1	43.1	7	43.0	1.8
	275	E11	50.7	44.5	50.0		39.0	42.1	29.8	42.7	7	42.7	2.7
	300	E12		47.0	51.8		41.4	41.5	34.8	43.8	6	43.4	2.3
	325	E13		50.9	54.0		43.4	42.5	38.9	46.5	6	46.0	2.3
	350	E14			59.8		43.9	35.1		47.4	4	46.5	5.1
	375	E15					47.6	40.5		43.9	3	44.0	2.1
	400	E16						40.5		40.5	2	40.5	0.0
	425	E17											
	450	E18											
	475	E19											
	500	E20											
		+1	54.1	60.8	66.5	56.4		44.4	50.0	55.9	7	55.4	2.7
		+2	55.0	62.8	68.1	47.3		51.1	67.3	58.5	7	58.6	3.0
		+5	54.7	67.1	70.3	50.7	59.0	52.3	73.6	60.8	8	61.1	3.0
		+10	53.8	64.0	62.7	51.1	55.0	47.4	73.5	57.9	8	58.2	2.9
		+20	46.6	49.9	49.0	40.9	45.4	42.7	57.1	47.1	8	47.3	1.8
		+30	42.2	42.6	42.1	41.6	38.1	40.8	46.5	41.6	8	41.9	0.8

Table B2.2.2 Blood data for RT for incremental exercise test ($[K^+]$)

RT VO2 peak test

$[K^+]$	Work-load		subject										
($mmol.l^{-1}$)	Watts	Sample #	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	3.67	3.77	4.01	3.94	4.01	4.15	4.07	4.08	8	3.96	0.06
	25	E1	3.80	3.85	4.03	3.98	4.20	4.17	4.17	4.33	8	4.07	0.06
	50	E2	3.89	4.04	4.05	4.14	4.37	4.31	4.22	4.46	8	4.19	0.07
	75	E3	4.01	4.10	4.24	4.24	4.48	4.37	4.29	4.44	8	4.27	0.06
	100	E4	4.19	4.22	4.40	4.36	4.62	4.31	4.43	4.58	8	4.39	0.05
	125	E5	4.37	4.34	4.45	4.45	4.75	4.41	4.48	4.61	8	4.48	0.05
	150	E6	4.46	4.44	4.60	4.57	5.05	4.35	4.54	4.63	8	4.58	0.07
	175	E7	4.63	4.58	4.62	4.65	5.07	4.45	4.62	4.61	8	4.65	0.06
	200	E8	4.74	4.84	4.60	4.81	5.23	4.45	4.61	4.72	8	4.75	0.08
	225	E9	4.89	5.09	4.79	4.92	5.49		4.62	4.98	7	4.97	0.10
	250	E10	5.08	5.43	4.93	5.21		4.68	4.67	4.87	7	4.98	0.11
	275	E11	5.21	6.51	5.21	5.54		4.92	4.82	5.89	7	5.44	0.22
	300	E12	5.37		5.50	5.74		5.09	5.37	5.98	6	5.51	0.13
	325	E13	5.79		5.92	6.35		5.19	5.37	6.35	6	5.83	0.20
	350	E14	6.13			6.27		5.51	5.45		4	5.84	0.21
	375	E15						5.99	5.83		2	5.91	0.08
	400	E16							5.88		1	5.88	
	425	E17											
	450	E18											
	475	E19											
	500	E20											
		+1	5.54	5.51	5.47	5.33	5.50		4.97	5.83	7	5.45	0.10
		+2	4.78	4.62	4.40	4.45	4.85		4.08	5.05	7	4.60	0.12
		+5	4.13	3.81	3.94	4.12	3.91	3.99	3.95	3.93	8	3.97	0.04
		+10	4.06	3.86	4.07	2.60	3.76	4.10	4.03	3.91	8	3.80	0.18
		+20	4.10	3.92	4.23	2.57	3.79	3.97	3.97	3.96	8	3.81	0.18
		+30	4.10	3.80	4.20	4.10	3.66	2.69	3.80	3.75	8	3.76	0.17

Table B2.2.3 Blood data for ET for incremental exercise test (blood [lactate] and [Hb])

ET VO2 peak test	Work-load		subject										
[bl lac] (mmol.l^{-1})	Watts	Sample#	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	1.10	1.09	0.46	1.00	1.21	1.23	0.66	1.39	8	1.02	0.11
	25	E1	1.20	1.24	1.30	1.19	1.21	1.39	1.07	0.55	8	1.14	0.09
	50	E2	1.19	1.43	1.26	1.31	1.20	1.37	1.02	3.80	8	1.57	0.32
	75	E3	1.14	1.40	1.12	1.50	1.24	1.74	1.00	1.23	8	1.30	0.08
	100	E4	1.24	1.39	1.10	1.39	1.39	1.76	0.87	1.40	8	1.32	0.09
	125	E5	0.97	1.47	1.13	1.13	1.47	2.39	1.16	1.29	8	1.38	0.16
	150	E6	1.00	1.43	1.47	1.78	1.54	2.82	1.20	1.56	8	1.60	0.19
	175	E7	1.07	1.53	1.47	1.80	1.81	3.46	1.36	1.85	8	1.79	0.26
	200	E8	1.03	1.39	1.80	2.10	2.28	4.21	1.49	0.45	8	1.84	0.40
	225	E9		1.98	1.88	2.86	2.47	5.91	1.50	2.18	8	2.68	0.56
	250	E10	1.26	2.21	1.88	3.39	4.44	7.57	1.99	2.03	8	3.10	0.73
	275	E11	0.79	2.54	2.25	3.97	5.75	10.88	2.65	3.76	8	4.07	1.10
	300	E12	1.56	3.69	2.96	4.71	7.78	13.48	3.12	3.25	8	5.07	1.36
	325	E13	1.63	4.11	3.75	6.60	7.95		4.14	1.60	7	4.25	0.89
	350	E14	2.00	4.61	4.54	7.58	9.93		6.53	1.91	7	5.30	1.11
	375	E15	2.58	5.18	5.07	10.27			7.85	2.74	6	5.61	1.22
	400	E16	2.03	8.15	8.12	10.53				11.52	5	8.07	1.65
	425	E17	4.41	9.91	8.66						3	7.66	1.66
	450	E18	5.86								1	5.86	
	475	E19	7.64								1	7.64	
	500	E20											
		+1	12.22	13.18	10.45	12.06	11.62		8.99	11.98	8	11.50	0.52
		+2	11.89	13.28	13.15	12.10	11.22	20.94	9.86		8	13.21	1.36
		+5	11.52	14.16	12.46	11.79	11.21	20.87	8.78	13.95	8	13.09	1.26
		+10	8.48	12.09	9.99	10.91	10.87	18.83	6.72	5.59	8	10.43	1.43
		+20	4.94	6.77	5.55	7.26	5.08	2.54	4.97	5.18	8	5.29	0.50
		+30	3.26	4.48	3.90	4.54	3.63	1.57	3.33	1.94	8	3.33	0.38
[Hb] ($g.dl^{-1}$)	Watts	Sample#	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	14.6	15.0	15.5	15.0	16.1	14.9	15.1	16.1	8	15.3	0.2
	25	E1	14.2	15.7	15.4	15.3	16.3	15.3	14.8	16.2	8	15.4	0.2
	50	E2	14.7	15.6	15.7	16.0	16.2	15.6	14.6	16.7	8	15.6	0.3
	75	E3	14.7	15.5	15.6	16.2	16.3	15.3	15.0	16.8	8	15.7	0.3
	100	E4	14.7	15.6	15.4	16.1	16.9	15.8	15.0	16.7	8	15.8	0.3
	125	E5	14.9	15.7	15.9	16.1	16.8	15.4	15.2	16.8	8	15.8	0.3
	150	E6	14.7	16.1	16.2	15.9	16.6	15.8	15.4	16.7	8	15.9	0.2
	175	E7	14.8	15.6	16.2	16.1	17.0	15.5	15.0	16.9	8	15.9	0.3
	200	E8	15.0	15.8	16.3	16.3	17.0	15.6	15.3	17.0	8	16.0	0.3
	225	E9	15.0	16.1	16.4	16.3	17.0	15.8	15.3	17.3	8	16.2	0.3
	250	E10	15.0	16.0	16.3	16.4	17.1	16.2	15.5	17.0	8	16.2	0.3
	275	E11		16.2	16.1	16.4	17.3	15.6	15.3	17.2	8	16.3	0.3
	300	E12		16.4	16.6	16.9	17.5	15.6	15.7	17.2	8	16.6	0.3
	325	E13	14.6	16.1	16.8	16.7	17.5	16.0	15.7	17.2	8	16.3	0.3
	350	E14	15.1	16.3	17.0	16.9	17.6		15.9	18.1	7	16.7	0.4
	375	E15	14.6	16.2	16.9	17.0			16.1	18.1	6	16.5	0.5
	400	E16		16.7	17.4	17.0				18.5	5	17.4	0.4
	425	E17		16.7	17.3						2	17.0	0.3
	450	E18		16.5							2	16.5	
	475	E19	14.8								1	14.8	
	500	E20											
		+1		16.7	16.9	17.6	17.8	16.6	15.9	18.5	8	17.1	0.3
		+2	16.2	16.5	17.1	17.4	17.9	16.6	16.0	18.5	8	17.0	0.3
		+5	15.8	16.7	16.3	16.3	17.2	13.8	15.6	17.6	8	16.2	0.4
		+10	15.2	16.7	15.7	16.1	17.1	13.8	15.0	17.4	8	15.9	0.4
		+20	14.3	16.2	15.8	15.7	16.4		14.9	16.3	7	15.7	0.3
		+30		15.9	15.9	15.3	16.0		14.8	15.8	7	15.6	0.2

Table B2.2.3 Blood data for ET for incremental exercise test (Δ plasma volume and $[H^+]$)

dPV	Work-load		subject										
(%)	Watts	Sample#	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8	0.0	0.0
	25	E1	10.4	-0.6	-0.6	-5.3	0.8	-9.3	-1.8	-5.9	8	-1.6	2.1
	50	E2	3.0	7.0	-3.7	-8.5	-0.9	-9.2	1.4	-9.6	8	-2.6	2.2
	75	E3	0.8	5.8	8.5	-11.1	-1.7	-4.9	0.5	-6.0	8	-1.0	2.2
	100	E4		11.7	1.9	-11.0	-1.0	-13.7	0.6	-8.9	7	-2.9	3.3
	125	E5	5.2	6.4	-2.1	-10.1	-3.7	-9.3	-2.6	-6.6	8	-2.9	2.2
	150	E6	1.1	2.3	-8.6	-10.7	-9.7	-11.3	3.2	-6.8	8	-5.1	2.2
	175	E7		0.5	-1.7	-9.5	-0.7	-6.3	0.5	-8.4	7	-3.7	1.6
	200	E8	0.7	-0.7	-6.1	-13.0	-4.7	-7.0	-0.8	-9.6	8	-5.2	1.7
	225	E9	12.3	0.2	-0.6	-9.6	-13.4	-8.8	2.2	-12.6	8	-3.8	3.1
	250	E10	-1.8	4.2	-4.8	-15.8	-9.3	-13.5	-3.1	-13.7	8	-7.2	2.5
	275	E11		-2.0	-1.5	-15.5	-11.5	-11.9	1.9	-12.0	7	-7.5	2.6
	300	E12		-1.9	-6.7	-17.3	-15.1	-13.0	-7.6	-10.6	7	-10.3	2.0
	325	E13	0.8	-3.8	-8.9	-16.0	-6.5	-14.6	-6.3	-11.0	8	-8.3	2.0
	350	E14	1.6	-4.4	-9.9	-19.6	-13.0		-7.6	-15.1	7	-9.7	2.7
	375	E15	0.2	-12.7	-11.9	-20.1			-9.8	-21.2	6	-12.6	3.2
	400	E16		-3.8	-14.9	-21.4					3	-13.3	5.1
	425	E17		-5.2	-13.0						2	-9.1	3.9
	450	E18		-7.2							1	-7.2	
	475	E19	1.5								1	1.5	
	500	E20											
		+1		-8.0	-12.0	-26.9	-16.2	-19.1	-2.4	-23.7	7	-15.5	3.3
		+2	-11.9	-5.1	-16.4	-25.2	-17.6	-21.4	-10.2	-19.3	8	-15.9	2.3
		+5			-11.6	-15.6	-13.3	-5.5	-9.3	-13.3	6	-11.4	1.5
		+10			-4.6	-18.4	-9.4	0.5	-4.9	-13.2	6	-8.3	2.8
		+20			-6.9	-6.8	-3.8		1.7	-5.6	5	-4.3	1.6
		+30			-2.2	-3.2	1.4		1.1	-2.7	5	-1.1	1.0
$[H^+]$	Work-load		subject										
($nmol.l^{-1}$)	Watts	Sample#	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	37.2	37.4	36.6	35.2	38.2	37.8	31.7	40.5	8	36.8	0.9
	25	E1	38.3	37.7	35.2	35.1	39.7	37.4	36.7	38.9	8	37.4	0.6
	50	E2	37.7	38.4	36.5	35.7	40.5	37.4	37.6	39.4	8	37.9	0.5
	75	E3	38.5	39.3	36.3	36.8	41.0	38.9	39.4	38.9	8	38.6	0.5
	100	E4	37.7	38.7	36.8	36.6	41.1	38.7	41.0	40.2	8	38.9	0.6
	125	E5	37.5	38.8	38.5	37.4	40.1	39.4	40.4	40.1	8	39.0	0.4
	150	E6	38.4	40.5	38.3	38.5	41.3	39.8	40.8	42.2	8	40.0	0.5
	175	E7	38.8	40.2	37.8	38.6	41.2	40.9	40.7	40.4	8	39.8	0.4
	200	E8	38.5	40.0	38.2	38.4	41.7	40.6	43.2	41.3	8	40.2	0.6
	225	E9	38.0	40.5	38.4	39.8	42.4	33.3	42.7	42.3	8	39.7	1.1
	250	E10	38.9	40.8	39.7	39.6	44.4	36.1	43.0	43.8	8	40.8	1.0
	275	E11		40.7	40.6	41.6	45.6	36.6	43.5	43.9	7	41.8	1.1
	300	E12		41.5	43.4	42.2	48.0	44.7	44.0	46.9	7	44.4	0.9
	325	E13	39.1	42.7	43.6	44.7	49.1	38.1	45.8	47.2	8	43.8	1.3
	350	E14	40.3	44.3	46.7	48.5	51.4		46.1	50.6	7	46.8	1.4
	375	E15	40.4	46.3	49.8	53.7			49.5	52.8	6	48.8	2.0
	400	E16		48.4	54.5	57.7				58.3	4	54.7	2.3
	425	E17	39.0	51.1	57.3						3	49.1	5.4
	450	E18	38.0	54.3							2	46.2	8.2
	475	E19	44.2								1	44.2	
	500	E20											
		+1		63.6	64.4	66.2	60.1	74.1	56.5	59.3	7	63.5	2.2
		+2	63.1	65.0	63.4	68.4	63.8	64.7	59.0	64.1	8	63.9	0.9
		+5	61.2	69.7	65.9	67.3	66.7	71.9	57.3	69.5	8	66.2	1.7
		+10	53.8	61.4	58.1	60.8	57.8	69.2	48.5	67.1	8	59.6	2.4
		+20	44.8	49.8	43.0	47.2	46.2		44.3	53.3	7	46.9	1.4
		+30	41.5	43.7	38.8	41.4	42.1		39.6	45.2	7	41.7	0.8
											0		

Table B2.2.3 Blood data for ET for incremental exercise test ($[K^+]$)

$[K^+]$	Work-load		subject										
(mmol.l^{-1})	Watts	Sample#	#1	#2	#3	#4	#5	#6	#7	#8	n	mean	SE
	0	R	4.41	4.19	3.67	3.77	3.89	3.84	4.45	3.83	8	4.01	0.11
	25	E1	4.42	4.26	3.70	3.90	3.97	3.89	4.49	3.99	8	4.08	0.10
	50	E2	4.77	4.38	3.81	4.25	4.15	3.99	4.60	4.18	8	4.27	0.11
	75	E3	4.78	4.44	3.88	4.34	4.29	4.16	4.81	4.34	8	4.38	0.11
	100	E4	4.84	4.53	3.84	4.41	4.35	4.24	4.84	4.58	8	4.45	0.12
	125	E5	4.98	4.46	4.13	4.70	4.43	4.26	4.82	4.66	8	4.55	0.10
	150	E6	4.91	4.49	4.24	4.65	4.41	4.36	4.94	4.61	8	4.58	0.09
	175	E7	5.02	4.72	4.27	4.78	4.50	4.43	4.92	4.75	8	4.67	0.09
	200	E8	5.12	4.81	4.26	4.73	4.56	4.47	5.02	4.62	8	4.70	0.10
	225	E9	5.07	4.75	4.34	5.03	4.63	4.73	5.13	4.72	8	4.80	0.09
	250	E10	5.19	4.88	4.45	5.10	4.75	4.87	5.17	4.78	8	4.90	0.09
	275	E11		5.03	4.53	5.23	4.86	5.18	5.34	4.88	7	5.01	0.10
	300	E12		5.20	4.67	5.20	5.08	5.64	5.57	5.12	7	5.21	0.12
	325	E13	5.53	5.32	4.82	5.57	5.35	5.76	5.81	5.41	8	5.45	0.11
	350	E14	6.78	5.48	5.42	5.75	5.63		6.19	5.36	7	5.80	0.19
	375	E15	7.83	5.47	5.68	6.03			6.44	5.85	6	6.22	0.35
	400	E16		5.83	6.09	6.35				6.28	4	6.14	0.12
	425	E17	6.84	6.13	6.22						3	6.40	0.22
	450	E18	7.98	6.83							2	7.4	0.57
	475	E19	7.89								1	7.9	
	500	E20											
		+1		5.63	5.42	5.96	5.27	5.61	5.77	4.78	7	5.49	0.15
		+2	6.00	4.62	4.53	4.53	4.23	4.32	4.69	4.66	8	4.70	0.19
		+5	5.05	4.66	3.47	3.51	3.51	4.27	4.21	3.77	8	4.06	0.21
		+10	5.62	4.63	3.45	3.62	3.48	4.00	4.14	4.03	8	4.12	0.26
		+20	5.32	4.58	3.55	3.71	3.59		4.24	3.89	7	4.13	0.24
		+30	5.31	4.53	3.62	3.70	3.65		3.98	3.79	7	4.08	0.24

Table B2.2.4 Blood data for LTx for incremental exercise test (blood [lactate] and [Hb])

LTx VO2 peak test	Work-load		subject										
[bl lac]	Watts	Sample #	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
(mmol.l^{-1})	0	R	0.7	1.3	1.6	1.3	2.3	0.8	2.4	2.0	8	1.6	0.2
	16	E1	0.9	1.4	1.9	1.5	2.7	1.0	2.4	2.2	8	1.8	0.2
	33	E2	1.1	1.7	2.7	2.1	3.6	1.5	2.8	2.7	8	2.3	0.3
	49	E3	1.6	2.7	3.9		5.0	2.2	3.4	3.4	7	3.2	0.4
	65	E4	2.1	3.8		4.3		3.4		4.5	5	3.6	0.4
	82	E5	2.9	5.5			6.1	4.4	5.6	5.5	6	5.0	0.5
	98	E6	3.8					5.8		6.8	3	5.5	0.9
	114	E7	5.4					8.4			3	6.9	1.5
	131	E8											
		E9											
		E10											
		E11											
		+1	7.3		6.4		8.1	10.7		8.6	5	8.2	0.7
		+2	8	6	6.5	5.1	8.6	10.8	8.7	8.9	8	7.8	0.7
		+5	7.9	6.2	6.1	4.7	8.4	10.3	8.9	8.9	8	7.7	0.7
		+10	7.1	5.7	5.5	3.9	7.7	9.1	7	7.7	8	6.7	0.6
		+20	5.2	3.9	4.2	2.6	5.6	6.3	3.9	5.5	8	4.7	0.4
		+30	3.3	2.6	3.1	2.1	4.3	4.1	2.8	3.9	8	3.3	0.3
[Hb]	Work-load		subject										
(g.dl^{-1})	Watts	Sample #	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	11.9	12.1	9.7	11.6	11.3	11.5	12.6	11.8	8	11.6	0.3
	16	E1	11.9	12.2		11.6	11.3	11.5	12.6	11.9	7	11.9	0.2
	33	E2	12.0	12.0	10.3	11.5	11.6	11.5	12.6	12.0	8	11.7	0.2
	49	E3	12.0	12.2	10.5	11.7	11.3	11.7	12.6	12.0	8	11.8	0.2
	65	E4	12.0	12.4		11.7		11.5		12.1	5	11.9	0.2
	82	E5	11.8	12.6			11.7	11.8	13.0	12.2	6	12.2	0.2
	98	E6	12.1					11.9	12.9	12.4	4	12.3	0.2
	114	E7	12.0					12.0			2	12.0	0.0
	131	E8											
		E9											
		E10											
		E11											
		+1	12.4		10.8		11.4	12.1		12.4	5	11.8	0.3
		+2	12.3	12.2	10.7	11.9	11.6	12.0	13.5	12.2	8	12.1	0.3
		+5	12.3	12.4	10.6	11.9	11.1	11.7	12.7	12.0	8	11.8	0.2
		+10	12.2	12.3	10.4	11.7	10.9	11.7	12.6	11.7	8	11.7	0.3
		+20	12.1	12.2	10.4	11.4	10.7	11.4	12.8	11.7	8	11.6	0.3
		+30	12.0	11.9	10.3	11.3	10.6	11.2	12.6	11.8	8	11.5	0.3

Table B2.2.4 Blood data for LTx for incremental exercise test (Δ plasma volume and $[H^+]$)

dPV	Work-load		subject										
(%)	Watts	Sample #	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8	0.00	0.00
	16	E1	0.00	-0.83	-4.91	0.00	-0.01	-0.01	0.01	-0.84	8	-0.82	0.60
	33	E2	-0.83	0.83	-5.83	0.87	-2.59	0.00	0.00	-1.67	8	-1.15	0.79
	49	E3	-0.84	-0.82	-7.63			-1.71	-0.01	-1.67	6	-2.11	1.13
	65	E4	-0.84	-2.42						-2.49	3	-1.92	0.54
	82	E5	0.85	-3.98				-2.54		-3.27	4	-2.24	1.07
	98	E6	-1.66					-3.37		-4.85	3	-3.29	0.92
	114	E7	-0.85					-4.18			2	-2.51	1.66
	131	E8											
		E9											
		E10											
		E11											
		+1	-4.04		-10.48		-1.23	-4.96		-5.19	5	-5.18	1.50
		+2	-3.27		-9.35		-2.58	-4.17		-3.28	5	-4.53	1.23
		+5	-3.26	-2.42	-8.49	-2.51	1.80	-1.70	-0.79	-1.67	8	-2.38	1.03
		+10	-2.47	-1.63	-6.72	-0.85	3.68	-1.70	0.01	0.86	8	-1.10	1.05
		+20	-1.66	-0.81	-6.73	1.76	5.62	0.89	-1.56	0.85	8	-0.21	1.25
		+30	-0.83	1.68	-5.82	2.65	6.60	2.68	0.00	0.01	8	0.87	1.26
$[H^+]$	Work-load		subject										
($nmol.l^{-1}$)	Watts	Sample #	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	41.5	37.6	32.6	39.6	38.0	36.1	39.6	37.8	8	37.9	1.0
	16	E1	41.6	38.1	33.3	39.4	37.2	36.3	39.9	37.4	8	37.9	0.9
	33	E2	42.0	39.2	33.7	40.4	39.9	36.7	40.0	37.6	8	38.7	0.9
	49	E3	43.0	40.0	35.6	41.2	39.8	38.0	40.3	38.5	8	39.5	0.8
	65	E4	43.7	40.6		43.7		38.4		38.5	5	41.0	1.2
	82	E5	44.0	40.6			39.6	38.9	42.7	39.5	6	40.9	0.8
	98	E6	45.8					40.0	42.8	40.4	4	42.2	1.3
	114	E7	46.5					40.2			2	43.3	3.1
	131	E8											
		E9											
		E10											
		E11											
		+1	49.1		40.0		42.4	46.9		43.8	5	44.4	1.6
		+2	51.2	45.2	41.6	44.7	45.0	49.1	47.6	44.8	8	46.1	1.1
		+5	55.2	46.3	39.3	45.1	46.1	52.5	47.4	45.9	8	47.2	1.7
		+10	52.0	45.0	39.1	43.6	47.4	48.9	42.1	43.9	8	45.2	1.4
		+20	45.8	40.3	33.9	41.0	42.9	41.8	39.1	41.3	8	40.8	1.2
		+30	43.8	38.9	33.8	40.6	41.4	38.6	35.9	38.4	8	38.9	1.1

Table B2.2.4 Blood data for LTx for incremental exercise test ($[K^+]$)

$[K^+]$	Work-load		subject										
(mmol.l^{-1})	Watts	Sample #	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	4.3	4.8	5.3	5.3	4.9	4.8	5.1	4.3	8	4.85	0.14
	16	E1	4.4	4.7	5.5	5.5	5.2	4.9	5.3	4.3	8	4.98	0.17
	33	E2	4.5	4.9	5.7	5.7	5.3	5.1		4.4	7	5.09	0.20
	49	E3	4.7	5.3		5.9	5.9	5.1	5.5	4.6	7	5.29	0.20
	65	E4	4.8	5.7		5.9		5.4			4	5.45	0.24
	82	E5	5.1	6.1				5.6	5.8	5	5	5.52	0.21
	98	E6	5.5					5.9	6.3	5.3	4	5.75	0.22
	114	E7	5.9					6.3			2	6.10	0.20
	131	E8											
		E9											
		E10											
		E11											
		+1	5.6		6			5.5		4.5	4	5.40	0.32
		+2	5.1	5	5.5	5.4	5.2	4.9	5.1	4.2	8	5.05	0.14
		+5	4.3	4.5	5.1	5.3	5	4.6	4.9	4.1	8	4.73	0.15
		+10	4.2	4.5	5.1	5.2	4.9	4.9	5.1	4.3	8	4.78	0.14
		+20	4.3	4.6	5.2	5.4	4.6	4.9	5.1	4.1	8	4.78	0.16
		+30	4.4	4.5	5.1	5.4	4.5	4.8	5.1	4.1	8	4.74	0.15

Table B2.2.5 Blood data for CON for incremental exercise test (blood [lactate] and [Hb])

CON VO2 peak test	Work-load		subject										
[bl lac]	Watts	Sample #	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
(mmol.l^{-1})	0	R	1.49	1.34	1.16	0.85	0.89	0.87	0.52	0.80	8	1	0.1
	25	E1	1.97	1.20	1.98	0.87		1.16	0.60	0.80	7	1	0.2
	50	E2	2.58	1.33	2.74	1.04		1.68	0.70	0.92	7	2	0.3
	75	E3	3.70	1.57	2.11	1.29		2.57	0.71	1.12	7	2	0.4
	100	E4	5.69	1.97	3.89	1.81		3.65		1.21	6	3	0.7
	125	E5	7.20	2.78	4.68	2.38	4.24	4.63	1.03	1.50	8	4	0.7
	150	E6		4.20	6.16	3.90	6.64	7.94	0.88	1.87	7	5	1.0
	175	E7		5.39		6.03			1.46	2.54	4	4	1.1
	200	E8		7.65		8.76	11.96		1.94	3.09	5	7	1.9
	225	E9		8.65					2.47	4.46	3	5.19	1.82
	250	E10							3.61	5.91	2	4.76	1.15
	275	E11							5.41	7.88	2	6.65	1.24
	300	E12							5.89	7.85	2	6.87	0.98
	325	E13							7.68	12.46	2	10.07	2.39
	350	E14								16.88	1	16.88	
	375	E15											
		+1	11.15	10.06	9.09	8.82	14.14	9.49	10.02	17.18	8	11.2	1.0
		+2	11.98	11.68	24.04	12.23	15.64	10.61	10.49	20.87	8	14.7	1.8
		+5	12.67	12.47	25.29	12.71	15.22	11.16	10.17	21.29	8	15.1	1.9
		+10	11.69	10.11	25.59	10.67	10.87	10.45	8.79	19.44	8	13.5	2.1
		+20	7.65		10.53	7.47	8.92	7.33	4.92	16.38	7	9.0	1.4
		+30	1.47	4.09	0.66	5.49		4.92	2.62	10.89	7	4.3	1.3
[Hb]	Work-load		subject										
(g.dl^{-1})	Watts	Sample #	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	12.5	13.6	13.5	13.2	15.5	13.7	14.2	14.4	8	14	0.3
	25	E1	12.9	13.9	13.6	13.7		13.5	14.6	14.2	7	14	0.2
	50	E2	13.1	14.0	14.5	13.7		14.2	14.4	14.7	7	14	0.2
	75	E3	13.5	14.1	14.7	13.8		14.3	14.7	14.6	7	14	0.2
	100	E4	13.7	14.3	14.0	13.9		14.6	14.8	15.0	7	14	0.2
	125	E5	13.9	14.9	14.4	14.0	15.0	14.6	14.8	14.9	8	15	0.1
	150	E6		14.7	14.8	14.4	16.7	14.8	14.9	15.0	7	15	0.3
	175	E7		14.5	14.8	14.4			15.2	14.9	5	15	0.1
	200	E8		15.1		14.6	17.3		15.2	15.2	5	15	0.5
	225	E9		15.3		14.8			15.3		3	15.13	0.17
	250	E10							15.4	15.6	2	15.48	0.07
	275	E11							15.4	15.7	2	15.55	0.15
	300	E12							15.7		1	15.70	
	325	E13							15.8	16.3	2	16.05	0.25
	350	E14								16.6	1	16.55	
	375	E15											
	400	E16											
	425	E17											
	450	E18											
	475	E19											
	500	E20											
		+1	14.2	15.2	14.9	14.8	16.9	15.2	16.1	16.8	8	15.5	0.3
		+2	13.8	15.2	15.0	14.7	17.1	14.9	16.0	16.9	8	15.4	0.4
		+5		14.3	13.7	14.3	17.3	12.8	15.8	16.2	7	14.9	0.6
		+10	14.4	14.2	13.6		17.0	13.8	15.7	16.1	7	15.0	0.5
		+20	14.4	13.9	14.1	13.3	16.5	13.4	14.9	15.6	8	14.5	0.4
		+30	13.8	13.9	13.5	13.5	16.3	12.9	15.0	15.3	8	14.3	0.4

Table B2.2.5 Blood data for CON for incremental exercise test (Δ plasma volume & $[H^+]$)

CON VO2 peak test

dPV (%)	Work-load Watts	Sample #	subject #1	#2	#3	#4	#5	#6	#7	#8	n	mean	SE
	0	R	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8	0	0.0
	25	E1	-3.3	-5.0	-2.7	-4.8		2.4	-2.3	1.0	7	-2	1.1
	50	E2	-4.1	-6.9	-8.4	-6.2		-5.3	-0.9	-2.1	7	-5	1.0
	75	E3		-5.7	-4.0	-7.2		-5.8	-4.4	-0.3	6	-5	1.0
	100	E4	-9.4	-9.5	-1.3	-5.6		-9.9	-6.2	-3.1	7	-6	1.3
	125	E5	-10.5	-8.6	-4.7	-7.2	4.4	-10.7	-4.9	-4.9	8	-6	1.7
	150	E6		-11.7	-8.9	-12.1	-11.7	-8.6	-6.7	-3.5	7	-9	1.2
	175	E7		-9.9	-7.3	-12.3			-7.1	-5.1	5	-8	1.3
	200	E8		-13.4		-11.2	-14.9		-7.1	-9.0	5	-11	1.4
	225	E9		-16.0		-13.4			-11.1		3	-13.49	1.40
	250	E10							-9.7	-10.1	2	-9.92	0.19
	275	E11							-11.3	-14.0	2	-12.64	1.32
	300	E12							-13.6		1	-13.63	
	325	E13								-4.5	1	-4.53	
	350	E14								-19.1	1	-19.05	
	375	E15											
	500	E20											
		+1	-17.1	-5.4	-12.5	-17.3	-10.8	-15.6	-17.9	-21.7	8	-14.8	1.8
		+2	-15.2	-12.6	-12.1	-14.5		-14.2	-17.9	-24.9	7	-15.9	1.7
		+5		-9.3	-4.1	-12.2	-18.0	1.1	-16.6	-20.4	7	-11.4	3.0
		+10	-18.8	-5.6	-3.4		-15.6	-6.7	-18.8	-19.5	7	-12.6	2.7
		+20	-13.8	-5.6	-5.2	-3.2	-12.6	-0.6	-12.1	-13.6	8	-8.3	1.9
		+30	-9.5	-4.6	-0.5	-3.7	-6.5	7.4	-12.0	-14.3	8	-5.5	2.4

$[H^+]$ ($nmol.l^{-1}$)	Work-load Watts	Sample #	subject #1	#2	#3	#4	#5	#6	#7	#8	n	mean	SE
	0	R	36.9	37.5	35.0	37.2	36.7	36.0	36.4	38.1	8	36.7	0.3
	25	E1	38.8	37.7	36.7	36.9		37.3	38.1	38.6	7	37.7	0.3
	50	E2	40.0	38.5	37.2	37.5		38.4	39.2	39.7	7	38.6	0.4
	75	E3	41.8	38.7	37.2	38.9		39.9	38.6	37.5	7	38.9	0.6
	100	E4	43.7	38.8	38.7	39.6		40.0	39.0	39.3	7	39.9	0.7
	125	E5	44.7	39.1	39.8	40.7	42.3	39.4	39.1	39.3	8	40.6	0.7
	150	E6		40.1	40.4	41.6	40.1	40.6	39.4	40.0	7	40.3	0.3
	175	E7		39.5	40.3	43.5			39.2	40.0	5	40.5	0.8
	200	E8		43.7		46.8	43.8		39.9	40.1	5	42.8	1.3
	225	E9		41.9		46.9			39.9	40.6	4	42.3	1.6
	250	E10							40.3	41.7	2	41.0	0.7
	275	E11							40.7	42.6	2	41.7	0.9
	300	E12							42.2	43.7	2	42.9	0.7
	325	E13							45.6	44.9	2	45.2	0.3
	350	E14								50.6	1	50.6	
	400	E16											
		+1	52.6	46.1	44.7	53.0	50.4	44.4	54.1	63.9	8	51.1	2.3
		+2	48.8	48.6	44.5	52.4	52.5	44.9	55.3	67.6	8	51.8	2.6
		+5	55.1	51.3	48.1	55.0	56.0	48.8	61.7	75.8	8	56.5	3.2
		+10	53.6	48.6	49.4	52.2	61.2	47.6	60.6	73.6	8	55.9	3.1
		+20	49.7	40.7	44.3	46.1	55.0	44.3	50.4	65.3	8	49.5	2.7
		+30	40.3	39.4	42.3	42.3	45.5	40.0	43.8	51.1	8	43.1	1.4

Table B2.2.5 Blood data for CON for incremental exercise test ($[K^+]$)

CON VO2 peak test

$[K^+]$	Work-load		subject										
($mmol.l^{-1}$)	Watts	Sample #	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**n**	**mean**	**SE**
	0	R	3.66	3.96	3.68	3.81	3.69	3.51	4.04	4.09	8	3.80	0.07
	25	E1	3.96	3.91	4.06	4.07		3.52	4.23	4.40	7	4.02	0.10
	50	E2	4.17	4.19	4.23	4.19		3.99	4.21	4.55	7	4.22	0.06
	75	E3	4.48	4.30	3.99	4.36		4.24	4.37	4.59	7	4.33	0.07
	100	E4	4.68	4.51	4.32	4.52		4.49	4.48	4.64	7	4.52	0.04
	125	E5	5.05	4.68	4.44	4.61	5.22	4.78	4.49	4.72	8	4.75	0.10
	150	E6		4.87	4.62	4.83		5.24	4.62	5.42	7	4.93	0.13
	175	E7		5.03	5.20	5.10			4.74	4.77	5	4.97	0.09
	200	E8		5.37		5.45	6.33		4.94	5.24	5	5.46	0.23
	225	E9		5.84		5.96			5.12	5.57	4	5.62	0.19
	250	E10							5.36	5.64	2	5.50	0.14
	275	E11							5.65	5.63	2	5.64	0.01
	300	E12							5.86	6.22	2	6.04	0.18
	325	E13							6.28	7.39	2	6.83	0.55
	350	E14								7.02	1	7.02	
	375	E15											
	400	E16											
	425	E17											
	450	E18											
	475	E19											
	500	E20											
		+1	5.16	5.24	4.86	6.77		4.83	5.52	6.60	7	5.57	0.30
		+2	4.10	4.59	4.42	4.86	4.47	3.93	4.70	5.74	8	4.60	0.19
		+5	3.89	3.74	3.37	3.79	4.22	2.94	4.09	4.74	8	3.85	0.19
		+10	3.99	3.89	3.67	3.89	3.61	3.38	3.96	4.56	8	3.87	0.12
		+20	3.99	4.18	4.05	3.89	3.78	3.38	3.92	4.58	8	3.97	0.12
		+30	4.01	4.04	3.76	3.76	3.94	3.28	4.08	4.31	8	3.90	0.11

Table B2.3 Plasma [K^+] response during the incremental test for UT, RT, and ET

K^+ response during VO2 peak test

UT	peak K^+	delta K^+	dK^+/work	peak WR	rest K^+	fall in K^+ 1 min	fall in K^+ 2 min
#1	5.72	1.70	12.42	325	4.03	0.88	0.87
#2	6.07	1.89	13.85	325	4.18	0.40	0.45
#3	6.64	2.85	24.32	300	3.80	0.72	0.92
#4	5.96	2.17	32.07	225	3.88	0.14	0.64
#5	5.65	2.00	14.62	325	3.66	0.23	0.51
#6	5.75	1.82	15.51	300	3.94	0.64	0.74
#7	6.28	2.25	16.45	325	4.04	0.76	0.79
#8	7.02	2.93	18.60	350	4.09	0.42	0.64
n	**8**	**8**	**8**	**8**	**8**	**8**	**8**
mean	**6.14**	**2.20**	**18.48**	**309**	**3.95**	**0.52**	**0.69**
SD	**0.49**	**0.46**	**6.60**	**38**	**0.17**	**0.27**	**0.17**
SEM	**0.17**	**0.16**	**2.33**	**13**	**0.06**	**0.09**	**0.06**

RT	peak K^+	delta K^+	dK^+/work	peak WR	rest K^+	fall in K^+ 1 min	fall in K^+ 2 min
#1	6.13	2.46	15.62	350	3.67	0.59	0.68
#2	6.51	2.74	27.68	275	3.77	1.00	0.95
#3	5.92	1.91	13.99	325	4.01	0.45	0.76
#4	6.27	2.33	14.79	350	3.94	0.94	0.91
#5	5.485	1.48	21.85	225	4.01	-0.01	0.32
#6	5.99	1.85	10.25	375	4.15		
#7	5.88	1.82	8.90	400	4.07	0.91	0.90
#8	6.35	2.27	16.63	325	4.08	0.52	0.65
n	**8**	**8**	**8**	**8**	**8**	**7**	**7**
mean	**6.07**	**2.11**	**16.21**	**328**	**3.96**	**0.63**	**0.74**
SD	**0.32**	**0.41**	**6.09**	**56**	**0.16**	**0.36**	**0.22**
SEM	**0.11**	**0.15**	**2.15**	**20**	**0.06**	**0.14**	**0.08**

ET	peak K^+	delta K^+	dK^+/work	peak WR	rest K^+	fall in K^+ 1 min	fall in K^+ 2 min
#1	7.89	3.48	12.21	475	4.41		0.95
#2	6.83	2.645	10.31	450	4.19	1.20	1.11
#3	6.22	2.55	11.11	425	3.67	0.80	0.85
#4	6.35	2.58	12.65	400	3.77	0.39	0.91
#5	5.63	1.74	11.05	350	3.89	0.36	0.70
#6	5.76	1.92	14.07	325	3.84	0.15	0.72
#7	6.44	1.99	11.06	375	4.45	0.67	0.88
#8	6.28	2.45	12.01	400	3.83	1.50	0.81
n	**8**	**8**	**8**	**8**	**8**	**7**	**8**
mean	**6.43**	**2.42**	**11.81**	**400**	**4.01**	**0.72**	**0.86**
SD	**0.70**	**0.55**	**1.19**	**50**	**0.30**	**0.48**	**0.13**
SEM	**0.25**	**0.19**	**0.42**	**18**	**0.11**	**0.18**	**0.05**

Table B2.4 Plasma [K^+] response during the incremental test for CON and LTx

CON	peak K^+	delta K^+	dK^+/work	peak WR	rest K^+	fall in K^+ 1 min	fall in K^+ 2 min
#1	5.05	1.39	61.78	125	3.66		0.48
#2	5.835	1.88	27.85	225	3.955	0.6	0.62
#3	5.2	1.525	36.31	175	3.675	0.34	0.39
#4	5.96	2.155	31.93	225	3.805		0.55
#5	6.33	2.64	48.89	200	3.69		0.93
#6	5.235	1.73	54.92	150	3.505	0.405	0.65
#7	6.28	2.245	16.45	325	4.035	0.76	0.79
#8	7.02	2.93	18.60	350	4.09	0.42	0.64
n	**8**	**8**	**8**	**8**	**8**	**5**	**8**
mean	**5.86**	**2.06**	**37.09**	**222**	**3.80**	**0.51**	**0.63**
SD	**0.68**	**0.54**	**16.68**	**80**	**0.21**	**0.17**	**0.17**
SEM	**0.24**	**0.19**	**5.90**	**28**	**0.07**	**0.08**	**0.06**

LTx	peak K^+	delta K^+	dK^+/work	peak WR	rest K^+	fall in K^+ 1 min	fall in K^+ 2 min
#1	5.9	1.6	0.00	114.33	4.3	0.3	0.4
#2	6.1	1.3	0.00	81.66	4.8		0.55
#3	5.7	0.4	0.00	32.66	5.3	-0.3	0.1
#4	5.9	0.6	0.00	65.33	5.3		0.25
#5	5.9	1	0.00	48.99	4.9		0.35
#6	6.3	1.5	0.00	114.33	4.8	0.8	0.7
#7	6.3	1.2	0.00	97.99	5.1		0.6
#8	5.3	1	0.00	97.99	4.3	0.8	0.55
n	**8**	**8**	**8**	**8**	**8**	**4**	**8**
mean	**5.93**	**1.08**	**0.00**	**82**	**4.85**	**0.40**	**0.44**
SD	**0.33**	**0.42**	**0.00**	**30**	**0.39**	**0.52**	**0.20**
SEM	**0.12**	**0.15**	**0.00**	**11**	**0.14**	**0.26**	**0.07**

Appendix B3 Comparison of data collected for incremental exercise test used in Chapter 7

Table B3.1 Ventilatory data for 3 subjects for comparison of incremental exercise tests conducted at Victoria University of Technology (VUT) and The Alfred Hospital (AH). t-tests revealed no significant differences between the variables measured at the two institutions.

		VUT				AH			
Time (min)	Workrate (Watts)	$\dot{V}O_2$ (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	0.55	0.49	6.06	20.78	0.47	0.65	5.22	26.9
1	25	0.97	1.13	10.68	43.36	0.77	0.91	8.55	31.87
2	50	0.88	1.42	9.68	58.22	0.88	0.97	9.82	35.83
3	75	1.04	1.25	11.51	50.87	1.09	1.16	12.07	43.83
4	100	1.31	1.49	14.45	59.37	1.17	1.14	13.03	42.53
5	125	1.49	1.57	16.49	59.24	1.54	1.49	17.08	55.67
6	150	1.89	1.6	20.92	54.93	1.57	1.53	17.48	56.17
7	175	2.21	2.14	24.39	70.52	1.87	1.81	20.8	65.23
8	200	2.38	2.23	26.26	70.84	2.03	1.9	22.53	63.97
9	225	2.88	3.1	31.81	93.97	2.26	2.11	25.07	64.37
10	250	3.09	3.58	34.17	110.04	2.51	2.46	27.85	73.23
11	275	3.3	4.16	36.43	144.1	2.73	2.9	30.37	88.47
12	300	3.36	4.34	37.16	191.1	3.09	3.35	34.36	104.37
13	325					3.52	3.98	39.07	144.43
14	350					3.89	4.25	43.18	193.35
Subject 2									
0	0	0.41	0.35	5.16	13.33	0.37	0.28	4.63	10.5
1	25	0.56	0.46	7.04	16.15	0.62	0.48	7.77	16.73
2	50	0.84	0.65	10.6	20.16	0.65	0.5	8.21	16.17
3	75	1.03	0.86	13.06	24.77	0.93	0.74	11.76	21.8
4	100	1.3	1.14	16.43	31.17	1.13	0.94	14.26	26.8
5	125	1.55	1.5	19.55	40.02	1.35	0.8	17.05	33.2
6	150	1.84	1.92	23.18	51.27	1.51	0.148	19.07	39.43
7	175	2.06	2.25	26.06	60.75	1.86	1.91	23.54	51.17
8	200	2.46	2.72	31.11	75.48	2.12	2.22	26.73	61.2
9	225	2.73	3.16	34.46	92.94	2.29	2.51	28.95	72.87
10	250	3.01	3.68	37.95	114.24	2.84	3.15	35.82	92.9
11	275	3.06	4.16	38.58	153.34	2.92	3.43	36.89	113.23
12	300					3.41	4.17	42.99	156.7
Subject 3									
0	0	0.3	0.31	2.92	15.42	0.3	0.28	2.92	12.6
1	25	0.63	0.48	6.18	17.55	0.68	0.51	6.68	17.8
2	50	0.88	0.67	8.58	21.95	0.88	0.7	8.6	21.63
3	75	1.08	0.86	10.61	26.62	1.09	0.89	10.71	25.9
4	100	1.38	1.13	13.52	33.54	1.27	1.07	12.42	29.73
5	125	1.6	1.4	15.69	38.73	1.54	0.88	15.08	35.23
6	150	1.91	1.76	18.77	45.27	1.72	1.54	16.82	40.5
7	175	2.25	2.2	22.01	55.63	2.01	1.91	19.67	49.83
8	200	2.57	2.63	25.22	65.83	2.24	2.21	22.01	57.27
9	225	2.91	3.07	28.52	76.97	2.46	2.46	24.1	63.37
10	250	3.07	3.44	30.07	89.65	2.78	2.85	27.27	74.5
11	275	3.56	4.38	34.94	124.28	3.1	3.31	30.43	87.6
12	300					3.36	3.71	32.9	103.6
13	325					3.67	3.97	35.9	114.4

Table B3.2 Blood data for 3 subjects for comparison of incremental exercise tests conducted at Victoria University of Technology (VUT) and The Alfred Hospital (AH). t-tests revealed no significant differences between the variables measured at the two institutions.

		VUT				AH			
Time (min)	Workrate (Watts)	HR ($b.min^{-1}$)	$[K^+]$ ($mmol.l^{-1}$)	bl [Lac]* ($mmol.l^{-1}$)	$[H^+]$ ($nmol.l^{-1}$)	HR ($b\ min^{-1}$)	$[K^+]$ ($mmol.l^{-1}$)	bl [Lac] ($mmol.l^{-1}$)	$[H^+]$* ($nmol.l^{-1}$)
Subject 1									
0	0	91	4.7		36.5	82	4.9	0.8	
1	25	126				93			
2	50	128	4.7		30.5	87	4.6	1.1	
3	75	126				95			
4	100	135	4.8		31.0	98	4.8	1.8	
5	125	138				110			
6	150	146	5.0		36.3	115	5	3.1	
7	175	159				126			
8	200	159	5.1		39.8	134	5.4	4.9	
9	225	171	5.4		39.7	145	5.4	6.6	
10	250	177	5.6		40.9	153	5.7	8.4	
11	275	182	6.0		42.3	161	6	10.8	
12	300		6.4		46.0	167	6.4	13.8	
13	325					173			
14	350					168			
Subject 2									
0	0	84	4.7		39.6	70	4.7	0.6	
1	25	130				79			
2	50	125	4.8		39.4	82	4.99	0.9	
3	75	125				86			
4	100	139	5.1		40.8	96	5.2	1.9	
5	125	150				104			
6	150	139	5.6		42.6	113	5.8	3.6	
7	175	156				122			
8	200	170	6.0		43.7	132	5.9	6.3	
9	225	170	6.1		46.0	142	6.2	8.2	
10	250	170	6.0		44.4	153	6.6	11	
11	275	172	6.7		48.0	163	6.8	13	
12	300					166			
Subject 3									
0	0	84	4.6		38.7	84	5.2	0.7	
1	25	90				90			
2	50	92	4.9		40.5	92	5.5	0.8	
3	75	97				97			
4	100	103	5.2		41.7	103	5.6	1.4	
5	125	110				110			
6	150	116	5.4		42.9	116	6	2.3	
7	175	119				119			
8	200	132	5.7		44.3	132	6.5	4.2	
9	225	144	6.0		46.0	144	6.3	5.9	
10	250	155	6.3		47.3	155	6.6	7.7	
11	275	162	6.9		48.4	162	7	10.5	
12	300	173				173			
13	325	177				177			

* due to technical difficulties, blood lac was not measured at VUT and plasma $[H^+]$ was not measured at AH.

Appendix B4 Muscle data collected from resting biopsies for UT, RT, ET, CON and LTx and fatigue biopsies for UT, RT and ET.

Table B4.1 3-*O*-MFPase activity for UT, RT, ET, at rest after muscle fatigue test.

Abbreviations: r, rest; f, fatigue; prot, pmol min^{-1} mg^{-1} protein; wet wt nmol min^{-1} g^{-1} wet weight; 5.5, 5.5mM stimulating $[K^+]$

UT	prot r	wet wt r	prot r 5.5	wet wt r 5.5	prot f	wet wt f	prot f 5.5	wet wt f 5.5
1	1295	227	1144	201	1295	1123	824	148
2	1232	224	833	152	1172	1127	794	133
3								
4	951	167	756	133	1395	1148	483	80
5	1172	203	811	141	1253	1159	754	128
6	1395	234	912	153	1095	1002	1021	159
7	1253	219			1232	1057	947	162
8	1095	170	855	133	951	790	902	159
n	7	7	8	6	7	7	8	7
mean	1199	206	885	152	1199	1058	818	138
SE	54	10	48	10	54	49	62	11
RT								
1		189		166		922	745	150
2	1119	172	687	106	1119	778	665	133
3	1342	270	929	187	1342	1135	1013	210
4	965	189	668	131	965	1311	1117	208
5	1167	179	971	149	1308	1089	430	72
6.0	1308	214	847	138	1564	1576	833	126
7.0	1564	303	952	185	1600	1558	1361	220
8	1600	313	1247	244	1167	813	1426	240
n	7	8	7	8	7	8	8	8
mean	1295	229	900	163	1295	1148	949	170
SE	88	20	74	15	88	110	122	21
ET								
1	1487	271	1237	226	1487	1416	1002	165
2	1158	212	864	158	1158	1001	835	163
3	1321	224	998	169	1321	1128	909	125
4	1313	220	955	160	1313	1523	1276	187
5	1350	243	1205	216	1350	1070	869	138
6	1388	297	1072	230	1388	1320	920	138
7								
8	1496	278	1006	187	1496	943	727	116
n	7	7	7	7	7	7	7	7
mean	1359	249	1048	192	1359	1200	934	148
SE	44	12	51	12	44	84	65	9

Table B4.1 3-*O*-MFPase activity for CON and LTx at rest.

Abbreviations: r, rest; f, fatigue; prot, pmol min^{-1} mg^{-1} protein; wet wt nmol min^{-1} g^{-1} wet weight; 5.5, 5.5mM stimulating $[K^+]$

CON	prot r	wet wt r	prot r 5.5	wet wt r 5.5
1	1041	187	669	120
2	1528	197	1358	176
3	1652	149	1283	116
4	1184	146	797	98
5	1115	161	933	135
6	962	129	551	74
7	1172	203	811	141
8	1095	170	855	133
n	8	8	8	8
mean	1219	168	907	124
SE	86	9	99	11
LTx				
1	1011	172	849	144
2	1785	304	1286	219
3	1677	226	1143	154
4	1129	200	888	157
5	1710	261	1550	237
6	1101	199	1010	183
7	1493	194	1206	157
8	1620	203	1173	147
n	8	8	8	8
mean	1441	220	1138	175
SE	110	15	80	12

Table B4.2 Na^+, K^+-ATPase content (pmol.g^{-1} wet wt) measured via ^3H ouabain binding site content for UT, RT, ET, CON and LTx at rest.

	UT	RT	ET	CON	LTx
	329	292	384	277	264
	375	211	379	330	229
	305	278	394	209	305
	329	259	351	207	265
	250	331	344	191	225
	275	364	355	219	214
	285	362	305	250	395
	302	318	344	302	334
n	8	8	8	8	8
mean	306	302	357	248	279
SE	14	19	10	18	22

Table B4.3 Muscle fibre type proportion and protein determination for UT, RT, ET CON and LTx at rest, protein also determined at fatigue for UT, RT, ET.

UT	Fast twitch (%)	Slow twitch (%)	Muscle protein rest (%)	Muscle protein fatigue (%)
1	47.7	52.3	13.6	17.5
2	36.3	63.7	14.5	14.9
3	62.8	37.2	17.7	20.2
4	48.7	51.3	12.6	12.8
5	64.2	35.8	15.1	15.7
6	56.4	43.6	17.8	16.2
7	59.3	40.7	16.7	17.4
8	46.4	53.6	16.0	17.8
n	8	8	8	8
mean	52.7	47.3	15.5	16.6
SE	3.4	3.4	0.7	0.8
RT				
1	33.1	66.9		20.1
2	33.8	66.2	15.4	20.0
3	59.5	40.5	20.2	20.7
4	43.5	56.5	19.6	18.6
5	58.7	41.3	15.3	16.8
6.0	35.9	64.1	16.3	15.1
7.0	67.3	32.7	19.4	16.2
8	64.8	35.2	19.6	16.9
n	8	8	7	8
mean	49.6	50.4	18.0	18.0
SE	5.1	5.1	0.8	0.7
ET				
1	67.5	32.5	18.2	16.5
2	65.0	35.0	18.3	19.6
3	61.3	38.6	17.0	13.8
4	77.0	23.0	16.7	14.7
5	69.8	30.2	18.0	15.8
6	73.2	26.8	21.4	15.0
7	76.7	23.3	16.7	19.5
8	48.5	51.5	18.6	16.0
n	8	8	8	8
mean	67.4	32.6	18.1	16.4
SE	3.3	3.3	0.5	0.8

CON	Fast twitch (%)	Slow twitch (%)	Muscle protein rest (%)
1	47.6	52.4	12.9
2	47.7	52.3	9.0
3	62.8	37.2	12.3
4	57.3	42.7	14.5
5	60.7	39.3	13.4
6	50.3	49.7	17.4
7	60.1	39.9	15.6
8	56.4	43.6	
n	8	8	7
mean	55.4	44.6	13.6
SE	2.1	2.1	1.0
LTx			
1			
2	31.6	68.4	17.0
3	34.7	65.3	13.5
4	15.7	84.3	17.7
5	16.2	83.8	15.3
6	3.2	96.8	18.1
7	23.3	76.7	13.0
8	50.3	49.7	12.5
n	7	7	7
mean	25.0	75.0	15.3
SE	5.8	5.8	0.9

Table B4.4 Muscle metabolites for UT, RT, ET at rest.

Values are expressed as mmol.kg^{-1} dry weight of muscle, except [H^+] (nmol.L^{-1}) and glycogen (mmol glucosyl units.kg^{-1} dry muscle)

UT	ATP	ADP	AMP	IMP	Lactate	Glycogen	PCr	Cr	[H^+]
1	24.22	2.06	0.11	0.1	10.99	446	91.7	32.5	64.6
2	22.31	3.34	0.11	0.03	6.53	645	90.1	45.4	60.3
3	27.71	3.16	0.2	0.04	5.02	451	105.4	44.0	74.1
4	22.8	2.22	0.09	0.09	9.82	433	83.2	44.4	77.6
5	24.74	3.16	0.16	0.09	10.82	579	93.9	34.5	75.9
6	29.16	2.3	0.07	0.07	7.13	445	99.2	53.0	64.6
7	23.12	3.19	0.22	0.09	6.07	409	91.8	47.9	57.5
8	23.63	2.74	0.11	0.11	7.93	344	90.8	34.6	72.4
n	8	8	8	8	8	8	8	8	8
mean	24.71	2.77	0.13	0.08	8.04	469	93.3	42.0	68.4
SE	0.87	0.18	0.02	0.01	0.80	34	2.3	2.6	2.7
RT									
1	31.2	3.05	0.07	0.15	10.64	555	108.5	49.7	83.2
2	30.1	2.8	0.09	0.03	6.64	563	109.4	38.7	74.1
3	23.08	2.57	0.06	0.15	12.19	513	101.3	34.6	69.2
4	28.12	2.89	0.07	0.21	11.26	337	104.4	42.0	60.3
5	24.32	3.66	0.18	0.01	5.3	625	89.3	43.3	91.2
6.0	25.71	4.57	0.29	0.1	13.2	471	105.6	36.2	66.1
7.0	24.32	3.75	0.19	0.05	7.98	404	118.7	43.3	69.2
8	27.43	3.99	0.08	0.04	9.63	520	101.2	63.5	72.4
n	8	8	8	8	8	8	8	8	8
mean	26.79	3.41	0.13	0.09	9.61	498	104.8	43.9	73.2
SE	1.03	0.24	0.03	0.03	0.98	33	3.0	3.2	3.5
ET									
1					8.76	457			52.5
2	22.11	2.67	0.19	0.1	2.94	572	76.6	48.2	60.3
3					9.74	416			61.7
4	23.16	2.63	0.37	0.04	4.89	517	76.8	55.8	58.9
5	30.78	4.02	0.35	0.09	7.33	579	89.2	31.1	74.1
6	23.41	2.67	0.63	0.07	4.75	370	78.3	50.9	66.1
7	23.67	3.26	0.26	0.11	8.97	642	87.0	62.4	72.4
8	21.65	3.71	0.08	0.09	5.83	527	85.9	45.8	79.4
n	6	6	6	6	8	8	6	6	8
mean	24.13	3.16	0.31	0.08	6.65	510	82.3	49.0	65.7
SE	1.37	0.25	0.08	0.01	0.86	32	2.3	4.3	3.2

Table B4.5 Muscle metabolites for CON and LTx at rest

Values are expressed as mmol.kg^{-1} dry weight of muscle, except [H^+] (nmol.L^{-1}) and glycogen (mmol glucosyl units.kg^{-1} dry muscle)

CON	ATP	ADP	AMP	IMP	Lactate	Glycogen	PCr	Cr	[H^+]
1	33.33	2.49	0.47	0.07	4.6	309	98.9	58.5	79.4
2	26.05	2.41	0.14	0.02	6.7	336	96.3	39.3	81.3
3	24.74	2.34	0.37	0.1	10.8	359	79.2	48.7	81.3
4	23.5	2.15	0.07	0.06	8.5	371	92.7	55.2	75.9
5	24	2.29	0.29	0.03	10.9	550	100.5	57.3	75.9
6	26.54	2.06	0.11	0.04	9.4	351	93.9	34.5	75.9
7	23.63	3.16	0.2	0.04	7.9	321	90.8	34.6	72.4
8									
n	7	7	7	7	7	7	7	7	7
mean	25.97	2.41	0.24	0.05	8.40	371	93.2	46.9	77.4
SE	1.30	0.14	0.06	0.01	0.86	31	2.7	4.0	1.3
LTx									
1									
2	27.52	3.84	0.13	0.31	17.2	446	118.5	51.7	91.2
3	19.88	2.76	0.07	0.17	15.8	645	106.3	74.2	89.1
4	18.41	2.32	0.05	0.28	14.7	451	92.5	37.2	91.2
5	18.7	2.76	0.06	0.15	12.8	433	97.0	56.5	74.1
6	21.51	2.82	0.14	0.5	14.5	579	98.3	54.4	93.3
7	22.32	2.74	0.17	0.23	20.0	445	77.8	47.2	87.1
8	21.5	2.43	0.2	0.23	18.7	409	87.3	64.1	81.3
n	7	7	7	7	7	7	7	7	7
mean	21.41	2.81	0.12	0.27	16.26	487	96.8	55.0	86.8
SE	1.16	0.19	0.02	0.04	0.96	33	5.0	4.5	2.6

Table B4.6 Muscle metabolites for UT, RT, ET at fatigue.

Values are expressed as mmol.kg^{-1} dry weight of muscle, except [H$^+$] (nmol.L^{-1}) and glycogen (mmol glucosyl units.kg^{-1} dry muscle)

UT	ATP	ADP	AMP	IMP	Lactate	Glycogen	PCr	Cr	[H+]
1	27.03	2.44	0.09	2.54	82.7	309	22.3	101.9	138
2	18.42	2.87	0.14	8.79	142.4	359	27.5	108.1	125.9
3	20.08	3.95	0.2	4.15	92.6	336	37.2	112.2	234.4
4	17.15	2.14	0.11	2.14	128.0	371	24.6	103.0	182
5	21.81	5.47	0.22	1.29	72.5	550	46.4	82.1	128.8
6	27.42	2.3	0.08	2.3	76.1	351	41.3	110.9	144.5
7	17.31	1.07	0.22	1.92	66.7	321	39.3	100.4	138
8	22.08	2.92	0.11	2.92	71.3	251	47.6	77.7	128.8
n	8	8	8	8	8	8	8	8	8
mean	21.41	2.90	0.15	3.26	91.6	356	35.8	99.5	152.6
SE	1.43	0.47	0.02	0.84	10.0	31	3.5	4.6	13.3
RT									
1	23.16	3.3	0.11	5.7	103.1	483	36.3	121.9	218.8
2	24.21	2.63	0.12	0.86	59.57	387	43.0	105.1	158.5
3	23.56	2.91	0.06	0.75	59.11	371	30.7	105.2	162.2
4	25.27	2.53	0.1	1.07	67.87	315	54.7	91.7	158.5
5	26.81	5	0.11	0.21	101.1	408	29.5	113.8	213.8
6.0	26.81	4.77	0.26	0.79	64.36	311	48.2	113.8	204.2
7.0	22.79	4.53	0.24	1.45	118.4	324	38.4	103.5	166
8	20.48	2.46	0.19	0.9	115.6	408	53.4	111.3	199.5
n	8	8	8	8	8	8	8	8	8
mean	24.14	3.52	0.15	1.47	86.2	376	41.8	108.3	185.2
SE	0.76	0.38	0.03	0.62	9.1	21	3.4	3.2	9.3
ET									
1					72.6	316			134.9
2	23.14	3.86	0.4	2.54	37.5	366	61.2	63.5	128.8
3					84.4	290			138
4	18.05	4.23	0.28	4.15	44.9	484	55.8	76.9	120.2
5	20.93	2.46	0.41	1.29	61.9	381	49.1	71.2	104.7
6	18.23	3.03	0.08	2.3	91.3	244	42.0	87.3	144.5
7	23.4	3.95	0.43	2.92	85.3	431	63.9	85.5	104.7
8	21.05	3.97	0.55	2.14	50.7	278	55.8	76.0	190.5
n	6	6	6	6	8	8	6	6	8
mean	20.80	3.58	0.36	2.56	66.1	349	54.6	76.7	133.3
SE	0.94	0.28	0.07	0.39	7.2	29	3.3	3.6	9.7