Exercise intensity and *in vivo* muscle contractile performance

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The Illustrations on the following pages have been omitted on request of the University –

Abbreviations

ACL	Anterior cruciate ligament
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
Ca^{2+}	Calcium
CNS	Central nervous system
Cr	Creatine
CS	Citrate synthase
CSA	Cross sectional area
CT	Continuous training
CYt. Ox	Cytochrome oxidase
DMD	Duchenne muscular dystrophy
E-C coupling	Excitation-contraction coupling
EMG	Electromyography
TFR	Torque frequency
FG	Fast glycolytic
FI (%)	Fatigue index
FOG	Fast oxidative glycolytic
GET	Gas exchange threshold
GME	Gross mechanical efficiency
H^+	Hydrogen ions
HFF	High frequency fatigue
HHb	Deoxyhaemoglobin
ICC	Intraclass correlation coefficient
In vivo	Measure performed within living tissue
IT	Interval training
K^+	Potassium
LDH	Lactate dehydrogenase
LFF	Low frequency fatigue
LSD	Long slow distance
LT	Lactate threshold
LT-$\dot{V}O_2$	Lactate threshold as a percentage of $\dot{V}O_2$ max.
Mb	Myoglobin

MHC	Myosin heavy chain
MVC	Maximal voluntary contraction
MVIC	Maximal voluntary isometric contraction
Na^+	Sodium
NAD	Nicotinamide adenine dinucleotide
NIRS	Near infrared spectroscopy
NIRSm	Near infrared spectroscopy of the muscle
Norm TFR	Normalised torque frequency
O_2Hb	Oxyhaemoglobin
PCr	Phosphocreatine
PCSA	Physiological cross sectional area
PPo	Peak power
PPo_{100}	100% peak power
PPo_{50}	50% peak power
RER	Respiratory exchange ratio
RTD	Rate of torque development
RR	Rate of relaxtion
$RR_{½}$	Half relaxation time
$\Delta RR_{½}$	Percentage change in half relaxation time
ΔRTD	Percentage change in rate of torque development
s	Seconds
SO	Slow oxidative
$SR\ Ca^{2+}$	Sarcoplasmic reticulum calcium ions
TRIMP	Training impulse
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}O_2$	Oxygen consumption
$\dot{V}_E$	Ventilation
$\dot{V}O_2\ max$	Maximal oxygen consumption
W	Watt
WAnT FI (%)	Fatigue index from the Wingate anaerobic test
WAnT FR	Fatigue rate from the Wingate anaerobic test
WAnT	Wingate anaerobic test

Figures

Tables

Acknowledgments

I would like to dedicate this thesis to my family and particularly to Jessica who has always been, and continues to be, there for me whilst waiting for me to come home and say 'I've handed it in'!

1 Introduction

1.0 Summary

The aim of this thesis is to better our understanding of how muscle contributes to exercise performance. Although many factors make up a performance, the focus of this work will be to study the interaction of muscle contractile characteristics to exercise. This Chapter is a general overview of factors influencing exercise performance and measures used to assess the impact of exercise interventions.

1.1 Components of performance

Figure 1.0 Framework for the training process proposed by Smith (2003) (reproduced with permission).

Performance of any activity is affected by many factors including social, psychological, biomechanical and physiological (Figure 1.0 Smith (2003)).

The focus of this thesis will be on the contribution of muscle performance. This area is itself multifactorial (Figure 1.1) and is affected by contributions from cardiovascular, neural and muscular systems.

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                    ┌──────────────────────────┐
                    │   MUSCLE PERFORMANCE     │
                    └──────────────────────────┘
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	Cardiovascular	Neural	Muscular
System	Blood supply	Neuromuscular control	Bioenergetics
	Capillarisation	Motor unit recruitment	Muscle contractile properties
Measures	Heart rate O$_2$ uptake NIRS	Electrical stimulation ⟷	Muscle biopsy Lactate
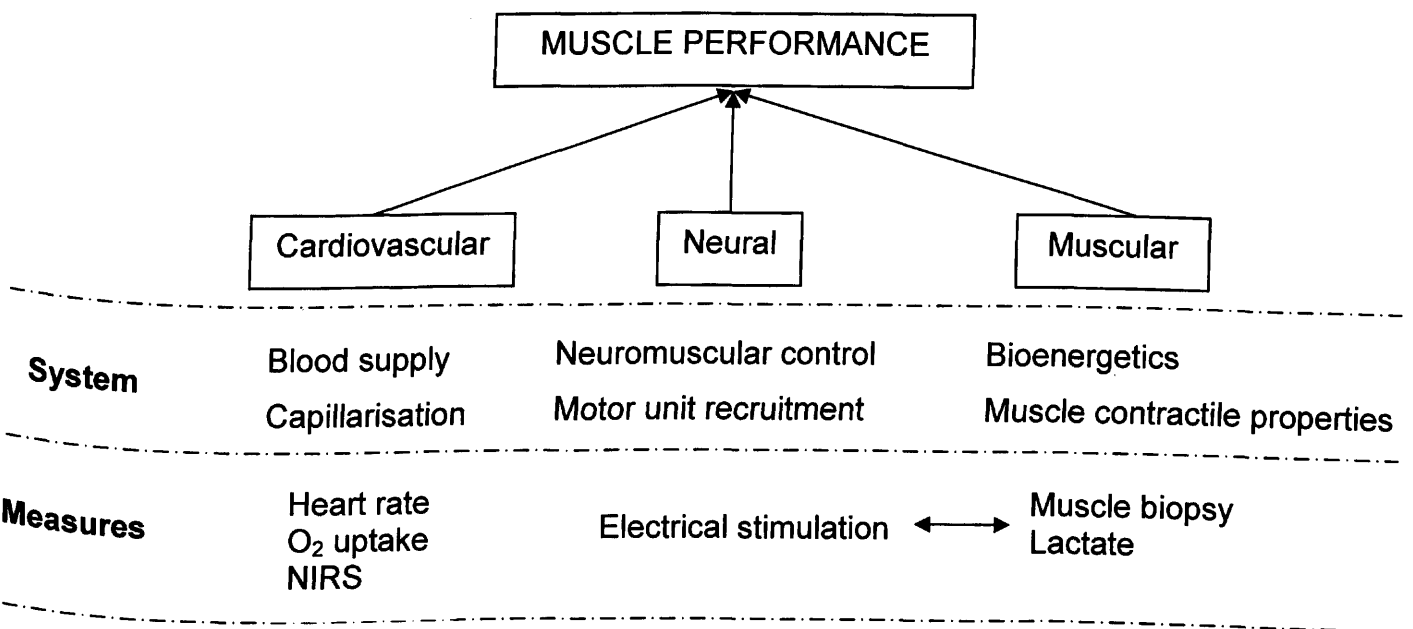
Figure 1.1 Physiological components contributing to performance

The degree of involvement of these systems depends on the task and the frequency, duration and intensity of which it is performed. An understanding of the interactions of the parameters displayed in Figure 1.1 is warranted, with particular emphasis on the requirements placed on skeletal musculature during various intensities of exercise.

1.1.1 Cardiovascular

There is much debate in the research literature on cardiovascular limitations to exercise (Bassett and Howley 2000; Noakes 2008; Saltin and Strange

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1992). It is beyond the scope of this thesis to discuss in detail the role played by the cardiovascular system on exercise performance, however a brief review of its impact on exercise performance is warranted.

Many believe, and there is good evidence to suggest, that cardiovascular limitations to exercise are central in origin (Bassett and Howley 2000; Dempsey 1986). Using a one-legged cycling model (Saltin 1985; Saltin *et al.* 1976) and single knee extension exercise (Mortensen *et al.* 2005) it is clear that the ability of the working musculature to utilise oxygen far outweighs the ability of the cardiovascular system to supply. In well-trained participants the over-development of the cardiovascular system can lead to exercise hypoxemia due to the increased cardiac output perfusing the lungs and therefore a reduction in arterial O_2 saturation from the reduction in mean transit time (Dempsey 1986).

Although central limitations are well documented, a crucial, often neglected, area of the cardiovascular system is the control of muscle blood flow via smooth muscle. Exercise training has demonstrated an improved vascular response to exercise (Lawrenson *et al.* 2003) and the level of control has varied depending on exercise intensity (Daussin *et al.* 2008). In addition to improved vascular control, significant increases in muscle capillarisation have been reported following training (Andersen and Henriksson 1977). A tighter control of blood flow, coupled with increased capillary density, allows greater O_2 diffusion at the capillary-muscle membrane. This is an important factor for maintaining metabolic control during exercise and is intrinsically linked to muscle bioenergetics (section 1.1.3).

1.1.2 Neural

The following section outlines both central and peripheral neuromuscular contributions to performance. Figure 1.2 highlights the integrative network of these systems and displays the possible sites where disruption within the network during exercise could lead to a reduction in task performance.

Figure 1.2 Possible sites of fatigue during exercise.

Performance can be affected by interruption or reduction anywhere along the pathway from central (points 1, 2 and 3 in Figure 1.2) through to the muscle contractile filaments (points 7 and 8 in Figure 1.2). There are a number of sites below the motor cortex which, if disrupted, which can lead to a reduction in performance. Exercise causing muscle damage has demonstrated a greater reduction in motor neuron excitability (point 3). Disruptions in the propagation of the action potential across the muscle fibre membrane (site 4)

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and into the muscle cell (site 5) have been reported following exercise (Fowles *et al.* 2002; Green 2004). One possible reason for the decreased excitability is alterations in potassium concentration that have been reported following exercise of sub-maximal (Leppik *et al.* 2004) and maximal (McKenna *et al.* 1993) intensity. The reduction in potassium levels can affect the excitability of the muscle membrane (point 4) (Kirkendall 1990; Sjogaard 1996). In a review by Clausen (2008) a number of studies demonstrated that high extracellular levels of potassium were strongly linked with force decline. The work also raised the issue of differences in potassium kinetics and force drop off between muscle fibre types. The rate of force drop off was six times more rapid in a fast twitch muscle compared to that of a slow twitch, and this was also reflected in a six-fold increase in extracellular potassium levels. Much work has been conducted assessing alterations in the excitation-contraction coupling failure (sites 7 and 8) following exercise (Green 2004; Green *et al.* 2004; Westerblad *et al.* 1998). Following fatiguing exercise, slow recovery of the processes involved with excitation-contraction coupling have been linked with the slow recovery of force following cessation of the exercise bout. Following a 4-minute fatiguing protocol, Ortenblad *et al.* (2000b) reported an 80% reduction in rate of force development and relaxation, with a significant reduction in sarcoplasmic reticulum calcium release following the intervention. Sarcoplasmic reticulum calcium levels remained reduced in the recovery phase in spite of the full recovery of ATP, phosphocreatine and lactate concentrations back to resting levels. This finding supports the theory that reduction in force output is not due solely to the build-up of metabolites (Allen *et al.* 2008). Disruption in sarcoplasmic reticulum calcium function has been reported in a number of studies as a major contributor to muscular

fatigue (Hill *et al.* 2001; Kirkendall 1990; Tupling *et al.* 2000; Vollestad and Sejersted 1988; Westerblad *et al.* 1998). The extent of the disruption varied depending on the duration, and particularly the intensity of the exercise (Leppik *et al.* 2004; Ortenblad *et al.* 2000a). Exercise intensity and duration place specific demands on the energy pathways and, depending on the training status of the participant, can have implications for performance outcome.

1.1.3 Muscle bioenergetics

The aim of the energy systems is to maintain adenosine triphosphate (ATP) homeostasis. Due to limited capacity to store ATP, there are three major energy pathways available for ATP resynthesis; adenosine triphosphate-creatine phosphate (ATP-CP), glycolysis and oxidative metabolism. ATP homeostasis is challenged by exercise and the predominant energy pathways for its resynthesis will vary depending on the intensity of the exercise bout. During high intensity bouts of exercise the majority of ATP resynthesis is via phosphocreatine (PCr) degradation and glycolysis. As displayed in equation 1.1 PCr donates its phosphate to ADP to resynthsise ATP.

$$PCr + ADP \xrightarrow{\text{Creatine kinase}} Cr + ATP$$
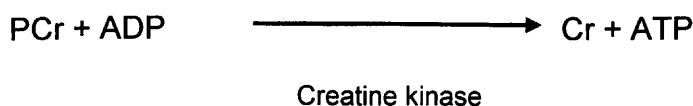
Eq. 1.1

The body can store approximately 70-80 mmol (kg of dm)$^{-1}$ of PCr and it is the predominant source for ATP resynthesis in approximately the first 10 seconds of exercise. This system for energy production is vital for explosive actions. For prolonged high intensity bouts a greater reliance is placed on glycolysis as

PCr stores become depleted. Glycolysis involves the degradation of glucose-6-phosphate to pyruvate and lactate. Crucial to the control of metabolism is the oxidisation of NADH back to NAD$^+$. In situations when the glycolytic flux exceeds mitochondrial activity, NADH is oxidised back to NAD$^+$ by the increased transfer of hydrogen ions (H$^+$) to pyruvate via the lactate dehydrogenase (LDH) reaction. This leads to the increased generation of lactate (Eq. 1.2).

$$\text{NADH} + \text{H}^+ \qquad\qquad \text{NAD}^+$$

Pyruvate $\longleftrightarrow$ Lactate

LDH
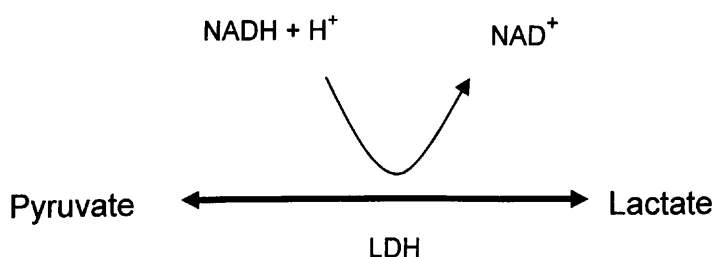
Eq. 1.2

The production of lactate is crucial for glycolysis to continue and is also a crucial substrate utilised in oxidative phosphorylation. However, prolonged high intensity work can lead to reductions in pH due to the accumulation of H$^+$ and the increased acidity can mean enzyme function could be compromised. The measurement of blood lactate is often used in exercise testing to give an indication of the energetic demand placed on the exercising musculature (Brooks *et al.* 2005). However, blood lactate concentration does not directly reflect that of the muscle, it only reflects the equilibrium of the metabolite. Throughout exercise, lactate is continuously generated (Figure 1.3), and can be utilised as a fuel source within the exercising muscle and at sites throughout the body by the reversal of the LDH reaction (Bergman *et al.* 1999). However, at increasing exercise intensities the ability to utilise lactate

is outweighed by its generation and ultimately concentrations in the blood increase. The point at which this imbalance occurs has been termed the lactate threshold (LT).

Figure1.3 Blood lactate response to incremental exercise (top panel) and its rate of appearance (R_a) and rate of disappearance (R_d) (bottom panel). (Reproduced with permission from Brooks *et al* (2005)).

For prolonged exercise there is a greater contribution from aerobic energy pathways, namely the Krebs Cycle and the electron transport chain within muscle mitochondria. In the presence of adequate mitochondrial activity and oxygen, pyruvate generated from glycolysis is converted to acetyl-CoA and enters the Krebs cycle for oxidation. Oxygen is not involved in the processes within the Krebs cycle but acts as a final hydrogen ion acceptor at the end of the electron transport chain. The H^+ generated in glycolysis, and via the enzymatic reactions in the Krebs cycle, are utilised to phosphorylate ADP for the further resynthesis of ATP. Fat as a substrate also enters the Krebs cycle

as acetyl-CoA to follow the process of oxidation. Individual training backgrounds have been shown to influence the contribution from energy systems to perform a particular task. The involvement of an energy pathway is also dependent on the type of muscle fibres recruited, which will be discussed in the following section.

1.1.4 Muscle contractile properties

It is clear that the duration and intensity of the exercise determines the contribution from particular muscle fibre types. Muscle responses are influenced by the type of muscle fibres recruited (Bottinelli and Reggiani 2000). It is generally accepted that muscle fibres are recruited voluntarily on the size principle (Henneman *et al.* 1965). During low intensity work, Type I muscle fibres are recruited first, then as intensity increases there is a greater involvement of Type IIa and then possibly Type IIx. This recruitment pattern is reflected in each muscle fibre type's energetic profile. Type I fibres are also known as slow oxidative (SO) fibres as they have a low peak force, slow relaxation time but possess high levels of oxidative enzymes. These fibres lend themselves to endurance exercise due to these characteristics. Type IIa, often classed as intermediate fibres, are also known as fast oxidative glycolytic (FOG) fibres. They possess high levels of both oxidative and glycolytic enzymes so have the ability to produce high force but have a better capacity to withstand fatigue. Type IIx, also known as fast glycolytic (FG) fibres, contain high levels of glycolytic, but low levels of oxidative, enzymes and therefore are recruited during short periods of extremely high intensity work as they fatigue very quickly. Also, within slow and fast muscle fibres different isozymes of LDH are found. In slow fibres, the LDH isozyme promotes the conversion of lactate to pyruvate (a shift from right to left in

equation 1.2) whereas both the fast fibre types contain LDH that promotes the conversion from pyruvate to lactate (Powers and Howley 1997).

1.2 General measures of responses to the training stimulus

As highlighted in the previous section, exercise intensity plays a key role in relation to the level of contribution from cardiovascular, neural and muscular systems. Equally as important when assessing the response to a training session is the duration of the exercise bout. Clearly exercise bouts of a high intensity cannot be performed over the same time periods as those of lower intensity bouts due to the metabolic requirements of such activities. A training stimulus is made up of frequency, duration and intensity (Zaryski and Smith 2005) and it is important that these are controlled to achieve the desired training outcome. It is vital that the athlete and coach utilise adequate measures for ensuring the correct training stimulus is applied for their specific event. A good measure should be valid (i.e. measure what is needed) and reliable (i.e. consistent over time). In the following sections a number of measures used to assess and quantify exercise intensity will be discussed.

1.2.1 Cardiovascular

1.2.1.1 Heart rate

The use of heart rate monitoring for the control of exercise intensity is common practice, simple to implement and is consistent from day to day (Lamberts *et al.* 2004). Heart rates are often linked with other physiological measures from laboratory exercise tests to prescribe appropriate exercise intensity (Jones 2007). Such physiological measures include the lactate threshold (Dumke *et al.* 2006), ventilatory and gas exchange thresholds (Lucia

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et al. 2000), $\%\dot{V}O_2$ max and specific powers or speeds. Work by Lucia *et al* (2000) demonstrated that heart rate values at the lactate and first and second ventilatory thresholds were stable throughout a season in an elite group of cyclists. Percentages of a subject's maximum heart rate can be utilised to construct training intensity zones aimed at improving a particular exercise goal. The American College of Sports Medicine's (ACSM) current position on the use of heart rate as a measure of exercise intensity states that the minimal training intensity for the development of $\dot{V}O_2$ max and lactate threshold, both seen as prerequisites of successful endurance performance, is at intensities between 55-65% maximum heart rate (ACSM 1998). These recommendations were directed towards healthy adults. Lounana *et al.* (2007) evaluated these findings in a population of elite endurance athletes. Their findings suggested that the ACSM guidelines underestimate the exercise intensities required for an elite endurance athlete. This highlights the issue that, when working with a range of subject populations, care must be taken when prescribing exercise intensity based on heart rate response alone. This is perhaps not surprising. It is common for an endurance athlete to display a lactate threshold of anywhere between 70-85% of $\dot{V}O_2$ max compared to 50-60% in untrained subjects. The metabolic responses would thus be vastly different between the subject groups even when performing at the same relative heart rate. Training at the heart rate zones recommended by the ACSM could therefore place greater strain on metabolic systems and the exercising musculature even at the same %HR between groups.

Heart rate responses have been successfully used to quantify the exercise intensity during sporting events and training (Lucia *et al.* 1999; Lucia *et al.* 2003; Padilla *et al.* 2000; Padilla *et al.* 2008). In a study to assess if any differences in exercise load was evident between two major cycle road races, Lucia *et al* (2003) used the heart rate response in an equation to calculate the training impulse (TRIMP score) for each of the races. A TRIMP score is based upon a heart rate value multiplied by the duration of the exercise (Banister 1991). This technique has proved useful in quantifying the exercise intensity placed on a competitor in a range of sports from individual to team events. The method has been developed from Banister's work by using different methods for factoring for the intensity of the effort (Foster *et al.* 2001; Lucia *et al.* 2003; Stagno *et al.* 2007). Although not a direct measure, this information can give an indication of the likely predominant energy systems and muscular strain during a performance.

1.2.1.2 Oxygen uptake

In the scientific literature a common method for setting the intensity of endurance training sessions is at percentages of a subject's maximal oxygen uptake (%$\dot{V}O_2$max) and other measures associated with this maximal measure e.g. velocity at $\dot{V}O_2$max (v$\dot{V}O_2$max). When using $\dot{V}O_2$ to quantify the metabolic demand of the exercise bout it is only a valid measure when ATP is resynthesised via oxidative phosphorylation. When exercising at higher intensities, such as above the lactate threshold, there is an increased energy contribution from non-oxidative pathways, such as glycolysis.

When using percentages of a subject's $\dot{V}O_2$max to set the exercise intensity for training, the majority of endurance training studies have utilised intensities

ranging between 60-75% $\dot{V}O_2$ max for continuous steady state training (Burgomaster *et al.* 2008; Gharbi *et al.* 2008) and during intermittent exercise work intensities have ranged from 80% to 175% $\dot{V}O_2$ max (Stepto *et al.* 1999). However, the findings on the most appropriate exercise intensity for optimal training adaptations are equivocal. Recent work has set exercise domains taking into account of other thresholds in relation to $\dot{V}O_2$ max (Wilkerson and Jones 2007). The rationale for this is that setting work rates at an arbitrary percentage of a subject's $\dot{V}O_2$ max does not take into account the differences in that individual's underlying metabolic responses. As mentioned with regards to working at %HR max, an exercise intensity of 60% $\dot{V}O_2$ max may be below one subject's lactate threshold but above that of another. The metabolic and muscle recruitment patterns in each case can thus vary greatly. For example, if subject 1's lactate threshold occurred at 70% $\dot{V}O_2$ max and they worked at a work rate corresponding to 60% $\dot{V}O_2$ max, their $\dot{V}O_2$ would reach a steady state, as their ability to supply the energy to complete the work can be met predominately oxidatively and with minimal involvement of Type II muscle fibres. The subject's pattern of $\dot{V}O_2$ would resemble that in Figure 1.4a in this case. In contrast, if a second subject had a lactate threshold at 50% $\dot{V}O_2$ max and also exercised at 60% $\dot{V}O_2$ max their pattern of $\dot{V}O_2$ would resemble that in Figure 1.4 c or d where there is a delay before a steady state is reached, if indeed it is reached at all, possibly due to greater Type II muscle fibre recruitment (Whipp 1994).

Thus when setting exercise intensity domains it is important that the subjects' $\dot{V}O_2$ max and sub maximal thresholds are taken into account, as this can have

profound effects on muscle fibre recruitment and contributions from energy pathways. This also allows equivalent metabolic responses to be applied to all subjects.

Figure 1.4 Oxygen uptake in one subject to four exercise intensities performed on a treadmill (o) and cycle ergometer (•). (A = 80% lactate threshold (LT); B = 25% of the difference between LT and $\dot{V}O_2$ max (25%Δ); C = 50%Δ; D = 75%Δ) (Data reproduced from Carter et al (2000) with permission).

Measurement of respiratory parameters such as ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) can give an indirect measure of aerobic and anaerobic energy production within the working musculature. During an incremental exercise test, where work rate is gradually increased, it is possible to identify the exercise intensity at which the subject can no longer supply the energy for exercise predominately from

aerobic sources but where there is an ever increasing contribution from anaerobic sources. This is evidenced by a disproportionate increase in $\dot{V}CO_2$ in relation to $\dot{V}O_2$ (Figure 1.5). The dashed line indicates the early linear response with the arrow indicating the point at which a greater proportion of energy is from anaerobic metabolism. At this point, to match the energy requirements of the exercise, glycolytic flux has increased above the point at which NAD can transport H^+ predominately to the electron transport chain. Therefore H^+, and consequently lactate concentrations increase. The increase in H^+ leads to a disproportionate increase in $\dot{V}CO_2$ in relation to $\dot{V}O_2$ due to the buffering of the H^+ via the bicarbonate system. This threshold has been termed the gas exchange threshold (GET) (Beaver *et al.* 1986) or metabolic threshold (Cooper and Storer 2001). It has been used frequently as a non-invasive measure of the lactate threshold and so the terms are often used synonymously.
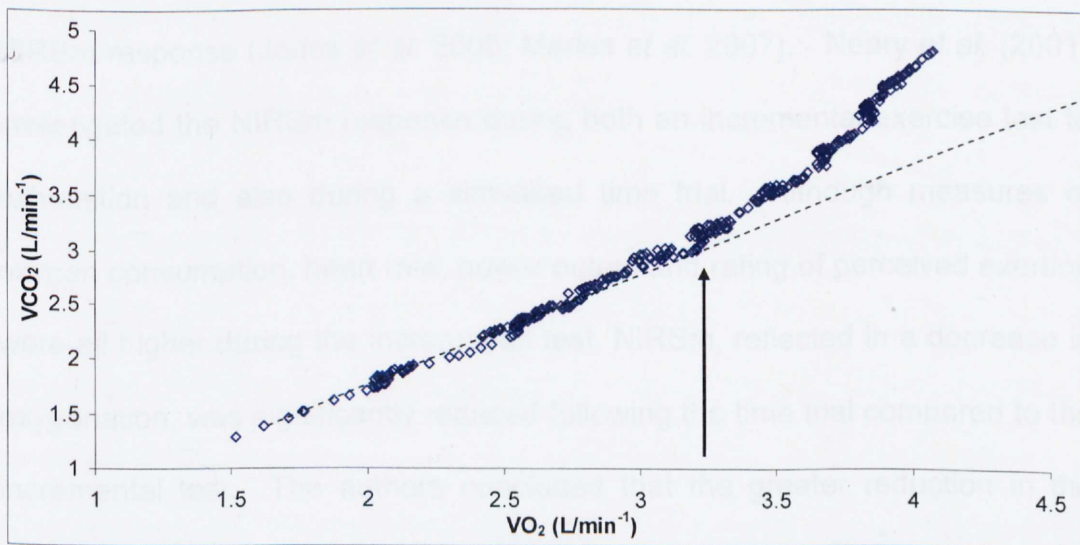
Figure 1.5 Gas exchange/metabolic threshold. (The arrow denotes the point at which there is a disproportionate increase in $\dot{V}CO_2$ to $\dot{V}O_2$) (Unpublished data M. Morris).

1.2.1.3 Near-infrared spectroscopy (NIRS).

Near-infrared spectroscopy (NIRS) is a non-invasive technique that can be utilised to monitor the degree of oxygenation and metabolism within a tissue. The technique works on the principle of light absorption, and within biological tissue at least three chromophores are measured; oxygenated haemoglobin (O_2Hb), deoxygenated haemoglobin (HHb) and cytochrome oxidase (Cyt.ox). Myoglobin (Mb) stores also affect the light absorbency, but have been shown to be very difficult to distinguish from that of Hb, so Mb is often included in these signals. Changes within these parameters can give an indication of oxygen utilisation, oxygen saturation and total haemoglobin (and therefore an indirect measure of blood flow) during various acute and chronic interventions. Muscle NIRS (NIRSm) has been shown to be sensitive at a range of exercise intensities ranging from above and below the lactate threshold (Belardinelli *et al.* 1995) and during maximal incremental exercise and simulated time trials (Neary *et al.* 2001). Priming exercise has also been demonstrated to alter the NIRSm response (Jones *et al.* 2006; Marles *et al.* 2007). Neary *et al.* (2001) investigated the NIRSm response during both an incremental exercise test to exhaustion and also during a simulated time trial. Although measures of oxygen consumption, heart rate, power output and rating of perceived exertion were all higher during the incremental test, NIRSm, reflected in a decrease in oxygenation, was significantly reduced following the time trial compared to the incremental test. The authors concluded that the greater reduction in the oxygenation response following the time trial was probably due to greater acidosis and possible changes in motor unit recruitment.

The NIRSm technique offers a sensitive measure of muscle oxygenation dynamics that could not previously be measured without the use of more

invasive methods. Further work is required to substantiate some of these interesting findings.

1.2.2 Muscle

1.2.2.1 Lactate

Lactate and lactic acid are terms often used interchangeably in the research literature. Lactate is formed from the disassociation of lactic acid and hydrogen ions (Brooks *et al.* 2005). Throughout this thesis the term lactate will be used.

Lactate has continually been linked with muscle fatigue and has been reported as a contributing factor for the loss of muscular force during exercise (Sahlin 1986). However, the link between lactate and fatigue has recently been questioned (Lamb and Stephenson 2006) as some studies have shown that high levels of lactate can be beneficial for muscle contractile performance (Nielsen *et al.* 2001), with others suggesting that lactate has a negative effect on muscle contractile performance (Favero *et al.* 1997). As already mentioned, lactate is also a vital substrate for energy production via oxidative metabolism.

The measurement of blood lactate levels throughout an incremental exercise test is often utilised to identify the point at which there is an imbalance in lactate appearance and disappearance rate. Training zones are often constructed from the measurements in relation to other physiological markers, such as heart rate (Figure 1.6) (Jones 2007). A number of specific points on the blood lactate response to incremental exercise have been identified as good markers for both training intensity and performance outcome (Bassett

and Howley 2000; Bentley *et al.* 2001; Dumke *et al.* 2006; Schabort *et al.* 2000).
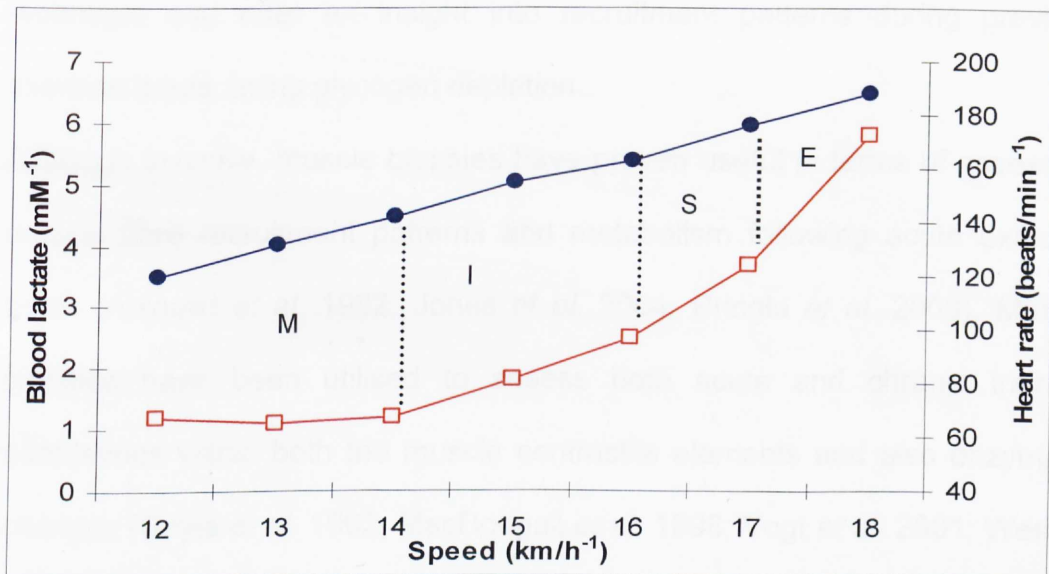
Figure 1.6 Construction of exercise domains from blood lactate (open squares) and heart rate response (filled circles) during an incremental treadmill test. (M= Moderate, I = Intense, S = Severe, E = Extreme) (Unpublished data M.Morris).

The first inflection point (in the example in Figure 1.6 this occurs around 14km/h^{-1}) has been linked with successful performance in the marathon (Jones 2006) and longer cycling time trials (Dumke *et al.* 2006). Performance outcome in shorter duration events has been linked with lactate levels around a second breakpoint in the lactate curve (in the example in Figure 1.6 around 16km/h^{-1}). As with the $\dot{V}O_2$ kinetics, exercise intensity plays a key role in the lactate response. The higher intensities require a greater involvement of Type II muscle fibres, showing an increased glycolytic contribution to energy supply.

1.2.2.2 Muscle biopsies

The muscle biopsy technique involves the extraction of approximately 0.5-1cm x 1mm of the muscle fibre. Fibre type proportions can be analysed based on myosin heavy chain (MHC) isoform (immunohistochemistry) or ATPase

activity (histochemical analysis). Using different assays this technique is also utilised to assess oxidative and glycolytic potential within the fibres. This technique can offer an insight into recruitment patterns during previous exercise bouts, using glycogen depletion.

Although invasive, muscle biopsies have proven useful in terms of assessing muscle fibre recruitment patterns and metabolism following acute exercise bouts (Ahlquist *et al.* 1992; Jones *et al.* 2004; Pringle *et al.* 2003). Muscle biopsies have been utilised to assess both acute and chronic training adaptations within both the muscle contractile elements and also enzymatic changes (Coyle *et al.* 1992; MacDougall *et al.* 1998; Vogt *et al.* 2001; Weston *et al.* 1997). Relating to the findings of Coyle *et al.* (1992) on the wide range of % Type I muscle fibres in a homogenised group, measuring an individual's muscle performance following a particular training bout could enable sessions to become even more individualised. Muscle biopsies have been utilised to assess muscle contributions during intermittent and continuous exercise bouts (Brickley *et al.* 2007; Palmer *et al.* 1999). Although conducted at a lower intensity than that of Brickley *et al.* (2007), Palmer *et al.* (1999) reported differences in fuel utilisation when comparing intermittent and continuous exercise bouts. Although the intermittent exercise was conducted at a lower intensity than that of Brickley *et al.* (2007), it was significantly longer. This study highlighted not only differing fuel utilisation between the bouts but also muscle fibre type dependency. Although there was no difference in total glycogen use between the bouts, following continuous exercise there was a greater reduction in glycogen content in Type I fibres, with a greater reduction in Type II fibres following the intermittent bout. This highlights the effect intensity plays in muscle fibre recruitment, as the total work completed was

the same. Dudley *et al.* (1982) reported the effects of intensity and duration on specific muscle fibre type adaptation. The work demonstrated the greater involvement of Type II fibres as exercise intensity increased. The greater glycogen depletion patterns in the Type II fibres in the work of Palmer *et al.* (1999) support this. Although not reported in the biopsies in more recent work of Burgomaster *et al.* (2005), the oxidative potential and subsequent improvement in a TTE test at 80% $\dot{V}O_2$ max is probably due to an improvement in the oxidative potential of the Type II fibres specifically.

1.2.3.3 Electrical stimulation

Stimulation of the muscle can be administered either through direct stimulation of the nerve (Millet *et al.* 2003) or from transcutaneous stimulation via electrodes placed directly over the muscle (Scott *et al.* 1990). Both methods record muscle contractile performance to allow non-invasive measures of fatigue resistance and contractile dynamics. Rutherford *et al.* (1986) compared direct stimulation of the nerve to transcutaneous stimulation and found no difference between the two techniques in muscle response. Stimulating the nerve innervating the muscle directly can be a painful procedure and therefore transcutaneous stimulation is often favoured. Stimulating the muscle directly allows assessment of muscular performance without motivational factors affecting the outcome. It is also a non-invasive technique for assessing muscle contractile characteristics. These are major advantages over other physiological measures. Early work by Edwards *et al.* (1977b) developed the technique for assessing muscle performance in clinical populations. This work described the characteristics of two different muscle groups and their response to stimuli of varying frequencies. Stimulating at low

frequencies (1-20Hz) through to high frequencies (40-100Hz) allows force-frequency curves to be established (Figure 1.7). These curves may then be used to assess muscle characteristics under varying conditions, such as preceding a fatiguing intervention.
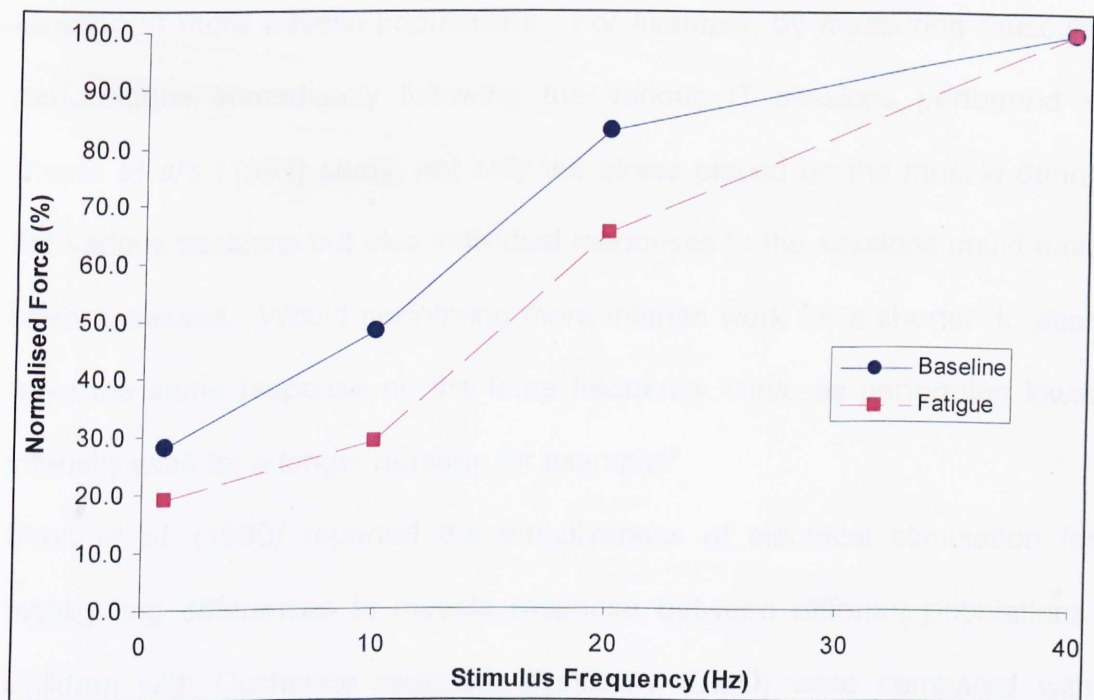
Figure 1.7 Example of force frequency curves before and after fatiguing protocol (unpublished data M.Morris).

Shields and Chang (1997) used force-frequency curves to assess the fatigue profile of paralysed soleus muscle. They measured the force-frequency on rested muscle immediately after administering a 2-minute fatiguing electrical stimulation protocol, and again after a 15-minute recovery period. Following the fatiguing protocol the force-frequencies displayed by the muscle had dropped significantly (by approximately 20% across the range of frequencies). This type of response is illustrated by the dashed line in Figure 1.7. Although their findings were related to developing optimal activation techniques for

paralysed muscle, the results also suggested that changes in force frequency curves could be used to assess muscular response following a fatiguing protocol or possibly an exercise intervention. The use of such force-frequency curves could shed light on the effect of particular exercise sessions on muscle function in more athletic populations. For example, by measuring muscular performance immediately following the various IT sessions performed in Stepto *et al's* (1999) study, not only the stress placed on the muscle during the various sessions but also individual responses to the sessions could have been assessed. Would performing more intense work for a shorter duration have the same response on the force frequency curve as performing lower intensity work for a longer duration for example?

Scott *et al.* (1990) reported the effectiveness of electrical stimulation for highlighting differences in muscle response between different populations. Children with Duchenne muscular dystrophy (DMD) were compared with healthy age-matched controls. The two populations were compared for fatigue resistance and half relaxation time of the muscle using electrical stimulation. Figure 1.8 displays a typical set of results from this experiment. The graphs display significant differences between the shapes of the graphs between normal and patients with DMD. Patients with DMD have been shown to demonstrate a selective degeneration in Type II muscle fibres (Webster *et al.* 1988). It could be assumed that at the start of the fatigue curve (figure 1.8 a) Type II muscle fibres in the stimulated area are recruited and once fatigued, force output comes predominately from Type I muscle fibres (Binder-Macleod and Snyder-Mackler 1993). This is an interesting theory as it highlights the possibility of using this technique to assess the effect of different training sessions on muscle fibre recruitment. If higher intensity sessions involve

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greater utilisation of Type II muscle fibres (Casey *et al.* 1996) then this could possibly be reflected in the shape of a fatigue curve immediately post-exercise. For example, following a supramaximal intermittent session, such as those conducted by Tabata *et al.* (1997), a healthy subject could demonstrate a post-exercise fatigue curve similar to that displayed by the DMD patient in figure 1.8b due to the fatiguing of Type II muscle fibres during the session. This hypothesis has yet to be examined.

Figure 1.8 Fatigue curves from normal (A) and Duchenne muscular dystrophy patients (B) (Reproduced with permission from Scott *et al.* (1990)).

The use of half relaxation times was another useful measure in differentiating the two groups in the study. The half relaxation time was calculated as the time taken for the muscle output to descend to half of the peak force, following an electrical stimulation. Normal healthy children displayed a mean half relaxation time of 111 msec before the fatigue test, and this time was elongated to 135 msec following the fatigue test. The DMD subjects showed no change in half relaxation times. This demonstrates that following a fatiguing protocol the electrical stimulation technique can detect changes in muscle contraction characteristics. The technique has displayed changes in muscle contractile characteristics following a controlled electrical fatigue

protocol, how they are altered following various exercise interventions, particularly of varying intensities, has yet to be explored. Non-invasive measurements, that can be repeated and assess the acute training response directly at the muscle level itself, would provide an invaluable additional tool for guiding training.

Electrical stimulation techniques were developed predominately in clinical settings, but more recently they have been utilised in more athletic populations. Acute and chronic training studies to assess whether training protocols had elicited changes within the muscle, or how athletes from differing backgrounds (such as endurance versus power sports) respond to training stressors at a muscular level, are becoming more frequent in the literature (Garland *et al.* 2004; Lepers *et al.* 2007; Skurvydas *et al.* 2002; Theurel and Lepers 2008).

Beelen *et al.* (1995) assessed the acute changes in both the stimulated and voluntary force muscle characteristics following short bouts of maximal exercise performed on a bicycle ergometer. This was one of the first studies to assess the effects of various durations of exercise bouts on muscle contractile characteristics. Longer duration maximal effort led to a greater decrement in both stimulated and voluntary force, but these had recovered by 3 minutes post exercise. The recovery of force followed a similar timescale to that of the resynthesis of phosphocreatine stores. One criticism is that of the control of intensity of the fatiguing cycle periods. Subjects were simply instructed to 'make a maximal effort for 25s'. There was no control on whether it was truly a maximal effort, bringing in large motivational variables. High blood lactate levels 20 minutes post exercise is evidence that the

subjects did apply great effort, but this level of 'maximal' effort could have varied greatly between subjects. With both stimulated and voluntary forces returning to baseline levels 3 minutes post-exercise, this suggests that high levels of blood lactate often linked synonymously with fatigue does not have a direct effect on muscular fatigue. Beelen *et al's* study was one of the few studies that recorded force output, both stimulated and voluntary, whilst their subjects remained on the ergometer. However, they did not measure any changes in fatigue characteristics following the exercise bouts. Measuring the subjects' muscle contractile characteristics such as rate of force and relaxation following the bouts could have highlighted individual responses to the varying lengths of work at the muscle level.

Skurvydas *et al.* (2002) measured muscle fatigue in differently trained subjects. Three distinct groups were tested; long-distance runners, sprinters and untrained. Each group was tested before and after completing 100 drop jumps at maximal intensity for maximal voluntary contraction, vertical jump height and electrical stimulation forces at 20 and 50Hz. Irrespective of training background, all of the groups showed a decrease in the selected measures. Most importantly, the drop in electrical stimulation force output at 20 and 50Hz was similar for all groups. All of the groups demonstrated a greater drop in the low frequency (20Hz) force output, termed low frequency fatigue (LFF). The eccentric action utilised in the drop jumps has been shown to predominantly damage fast twitch fibres (Byrne *et al.* 2004) therefore the fatigue recorded could not be attributed to differing muscle fibre composition, as the sprinter group should have demonstrated a greater reduction. However, Coyle *et al.* (1992) found that the percentage of Type I muscle fibres ranged from 32 to 76% in a homogenised group of cyclists and so it

may not be accurate to presume specific fibre type damage without muscle biopsies. More prolonged cycling exercise to that of Beelen *et al.* (1995) has demonstrated weakness within the muscle for up to six hours post-exercise (Bentley *et al.* 2000). The exercise bout involved a mixture of continuous and intermittent exercise efforts. The effects of these different modes of exercise on muscle contractile characteristics are presently unknown. It is known that, following running exercise, various areas of muscle contractile performance have been affected following continuous (Skof *et al.* 2006b; Place *et al.* 2004; Millet *et al.* 2004) and intermittent (Skof *et al.* 2006a) exercise. Muscle recruitment patterns differ between running and cycling and therefore the muscle responses should not be interpreted across modes. Recent work by Theurel *et al.* (2008) and Lepers *et al.* (2007) used direct nerve stimulation to assess continuous and intermittent exercise in cycling. Theurel *et al.* (2008) reported greater reductions in muscle contractile performance following intermittent exercise whilst Lepers *et al.* (2007) reported no difference between the exercise bouts. The discrepancy in these findings is presumably related to the wider range of exercise intensities used between the modes in the later study. To date no work has reported differences in muscle contractile performance following intermittent and continuous cycling exercise using direct muscle stimulation. It is clear that intensity plays a key role in the response to exercise. As yet, involvement of the muscular system to varying intensity of exercise is not fully understood.

Many studies have been conducted relating muscle characteristics to physiological and performance measures (Coyle 1999; Coyle 2005; Hawley 2002; MacDougall *et al.* 1998). Of these, only one (Garland *et al.* 2004) has assessed the relationship between *in vivo* muscle characteristics, measured

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using electrical stimulation, and other physiological measures. Clearly further work is required to increase our knowledge of the relationships between *in vivo* muscle characteristics and other physiological measures.

1.3 Types of training for endurance events

The type of training involved with developing the cardiovascular, neural and muscular systems can vary from almost purely aerobic, where the participant maintains a steady work output for prolonged periods or, from increased sources of anaerobic energy production, involving the participant to engage in high intensity periods of work, often interspersed with rest periods before repeating. The coordinated ability of energy and cardio-respiratory systems to respond and supply energy to meet demands placed on the exercising muscle and for the muscle to utilise supply during exercise is essential for successful performance. The ability of the human body to adapt to exercise stressors is considerable. Of particular interest is the role intensity plays in causing such adaptations. Tabata *et al.* (1997) demonstrated that the loading placed on the aerobic and anaerobic energy systems was dependent on the intensity and duration of the exercise bout. Improvements in performance can be obtained following very different training regimes (Burgomaster *et al.* 2006; Burgomaster *et al.* 2005; Daussin *et al.* 2008; Gibala *et al.* 2006; Stepto *et al.* 1999). It is evident that the intensity of exercise can result in dramatic and rapid adaptations in muscle energetics. However, how these alterations affect *in vivo* muscle performance characteristics after different intensities of exercise has yet to be established.

1.3.1 Long slow distance (LSD) training

Long, slow continuous exercise bouts, i.e. sessions that are maintained at low work intensities for a prolonged period of time, place particular physiological strain on aerobic energy production. This type of training is seen to be crucial for developing an 'aerobic base' fitness for the endurance athlete (Whyte 2006). Often this type of training involves completing distances far greater than that covered in competition. This type of prolonged training has been seen to be crucial for the development of key systems involved in aerobic metabolism. Early adaptations to LSD type training include a reduction in glycogen utilisation and lowered muscle lactate concentrations (Green et al. 1992) and decreased heart rate (Almeida and Arajo 2003). No improvements were noted in $\dot{V}O_2$ max or muscle mitochondria capacity. More prolonged training periods (>4 weeks) have reported increases in mitochondrial enzyme content (Gollnick et al. 1973; Holloszy 1967; Holloszy and Coyle 1984), increased $\dot{V}O_2$ max (Hurley et al. 1984), increased blood volume (Convertino 1991) and increased capillary density (Schantz et al. 1983). The magnitude of improvements will depend on the starting training status of the subject (Laursen and Jenkins 2002).

Harber et al. (2004) demonstrated alterations in specific muscle fibre type measures following a period of LSD. Peak force increased in both Type I and IIa fibres following a 12-week endurance training intervention, consisting of predominately LSD training. Maximal shortening velocity and fibre diameter decreased in Type I muscle fibres, with no change in Type IIa, demonstrating the fibre type specific adaptations to this prolonged LSD training.

1.3.2 Interval training

Interval training (IT) sessions, defined as high intensity work interspersed with rest periods, have been shown to stress both aerobic and anaerobic energy production systems (Tabata *et al.* 1997). However, the amount of stress placed on each energy system is very much dependent on the duration of the work and rest periods of the intervals (Tabata *et al.* 1997). The length of the exercise and the recovery periods have a significant effect on the aerobic contribution to high intensity exercise (Balsom *et al.* 1992) and there has been a range of work:rest ratios utilised within the research literature. Exercise interventions that incorporate periods of high intensity work interspersed with rest periods have been shown to elicit a more effective training stimulus than exercise conducted at a lower intensity without rest periods (continuous training) (Daussin *et al.* 2008; Helgerud *et al.* 2007). Exactly why this is has not been conclusively answered. However, the adaptations reported following IT are many and have included increased muscle oxidative and glycolytic enzymes (Burgomaster *et al.* 2005; Daussin *et al.* 2008), increased muscle buffering capacity (Weston *et al.* 1997), decreased reliance on carbohydrate oxidation (Westgarth-Taylor *et al.* 1997) and significant improvements in $\dot{V}O_2$ max (Helgerud *et al.* 2007; Laursen *et al.* 2002; Tabata *et al.* 1996). Performance trials such as a 40km time trial (Stepto *et al.* 1999) and time to exhaustion tests (Daussin *et al.* 2008) have demonstrated significant improvements following a period of IT.

As yet there is no universal measure to monitor muscle response following the various IT interventions employed in the research literature. Likewise, acute muscle performance measures following the training sessions in all of the studies mentioned could have highlighted the different responses between the

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training stimuli. How the various intensities affect *in vivo* muscle contractile characteristics is still unknown.

1.4 Summary

As illustrated by the findings of the previous studies, the adaptations to a training stimulus are many and vary depending on the subjects' training status. To date there is no universal measure that conclusively represents muscle performance changes, both acutely and chronically, following a training session or period of training, to aid the identification of the training stimulus placed on an individual at the muscle level.

The objective of this thesis is to examine the relationship of muscle performance to other physiological measures and to assess the effects of training intensity on muscle performance parameters. Although studies have utilised electrical stimulation techniques to assess muscle performance, work is still required in developing this technique alongside other physiological measures to assess how muscle performance data relates to a subjects training status and how it can inform regarding an individual's response to a range of exercise intensities. In order to complete this work the following will be performed:

1. Develop a direct muscle electrical stimulation technique and analysis method for the assessment of muscle contractile function (Chapter 3).

2. Explore the relationship of muscle contractile function to laboratory measures of anaerobic and aerobic performance (Chapters 4 and 5).

3. Explore the effects of high intensity exercise bouts on muscle contractile function (Chapter 6).

4. Examine the muscle response to sessions of different exercise intensities. (Chapter 7).

2 Quadriceps femoris

2.0 Summary

The majority of the training studies discussed in Chapter 1 involved the quadriceps femoris as the main muscle utilised during the exercise regime. Due to the importance of this muscle group, on not only sporting but everyday activities, the aim of this Chapter is to provide an anatomical background to the quadriceps femoris muscle group and an appreciation of its structure:function relationships.

2.1 Introduction

Many studies have been conducted to assess the quadriceps femoris in a clinical setting (Edwards *et al.* 1977b). The volume of work conducted on the muscle group highlights the importance of the quadriceps for daily functions such as walking, climbing stairs and squatting. The importance of the quadriceps in sporting activities has led to much work being conducted on assessing its function in differently trained athletes (Garrandes *et al.* 2007), following various acute training sessions (Green *et al.* 2000; Skof and Strojnik 2006a; Skof and Strojnik 2006b; Theurel and Lepers 2008) and following more prolonged training interventions (Sinacore *et al.* 1994). This body of work also emphasises the key role this muscle group plays in sport.

The mode of exercise monitored throughout this thesis is cycle ergometry. The quadriceps is the main driver for this type of exercise and for this reason a detailed explanation of its relevant function and structure is warranted.

2.2 Gross anatomy of the quadriceps femoris group

The quadriceps group comprises four main muscles; rectus femoris, vastus lateralis, vastus medialis and vastus intermedius (Figure 2.0). Nerve and blood supply to the muscle group is from the femoral nerve and artery respectively. The position and function of the individual muscles, and their neural and blood supply, are described in the following sections.

Figure 2.0 The quadriceps femoris muscle group (Illustration by R. Thornley with permission).

1 Vastus lateralis

The lateralis is the largest muscle in the quadriceps group. The origin is on the greater trochanter and the muscle inserts onto the quadriceps tendon and the lateral surface of the patella. Its main role is to extend and stabilise the knee joint. The muscle fibres are unipennate to the aponeurosis. The blood supply is via the superior medial artery.

2 Rectus femoris

The origin of the rectus femoris is on the ilium and the muscle tapers distally into the quadriceps tendon. Its main role is flexion and extension of the hip

and knee joints. The muscle fibres have a superficial bipennate arrangement, with the deeper fibres more parallel to the aponeurosis. The main blood supply is via the profunda femoris artery.

3 Vastus intermedius

The intermedius is almost completely covered by the other muscles within the quadriceps group. Its origin is on the anterior and lateral surfaces of the femur and its insertion is onto the deeper portion of the quadriceps tendon. The blood supply is via the profunda femoris artery medially and laterally via the lateral circumflex artery.

4 Vastus medialis

The medialis sits on the medial aspect of the anterior thigh. The origins are from the intertrochanteric line and the tendons of the adductor muscles. It tapers distally into the quadriceps tendon and to the medial aspect of the patella. The muscle fibres are unipennate. The main role of this muscle is to extend and stabilise the knee joint. Muscle blood supply is directly from the superficial femoral artery.

As a functional group the different muscle components have various functions. On contraction, the rectus femoris pulls the patellar tendon along the line of the mechanical axis due its anterior positioning across the hip joint. The vasti muscles (i.e. the vastus lateralis and vastus intermedius) are attached to the shaft of the femur and therefore exert forces both laterally and proximally during contractions. The vastus medialis plays an important role in resisting lateral displacement of the patella during knee motion. In some cases it has been reported that there is fusion of the intermedius and medialis muscle and

this could have profound effects on both the function of the muscle and thus interpreting muscle activity data (Willan *et al.* 2002).

The nerve supply to the quadriceps muscle group is from the femoral nerve (Figure 2.1). Many studies have directly stimulated this nerve to activate the quadriceps muscle by pressing a stimulating electrode into the femoral triangle (Lattier *et al.* 2004; Lepers *et al.* 2001; Theurel and Lepers 2008). When stimulation of the quadriceps is conducted via surface electrodes placed directly over the muscle belly (Scott *et al.* 1986; Skurvydas *et al.* 2007), both the muscle and branches of the femoral nerve are directly activated.

Figure 2.1 Location of femoral nerve and artery. (Illustration by R. Thornley with permission)

When stimulating the muscle directly it has been reported that the muscles are recruited in a reverse order to that of voluntary activation (Hamada *et al.* 2004; Sinacore *et al.* 1990). One reason for this hypothesis is that the axons supplying the faster contracting fibres are more easily recruited during

electrical stimulation and are also situated more superficially, and thus closer to the electrodes. Other studies report that electrical stimulation does not necessarily recruit in a reverse order but a more synchronous manner to that of voluntary activation (Gregory and Bickel 2005). These theories on fibre type recruitment patterns are still debated in the research literature. The electrode placement, commonly used in the research literature, when stimulating the quadriceps is via two electrodes, one placed distally over the muscle belly of the vastus medialis and the second placed proximally on the anterolateral border leading to activation of the vastus lateralis and rectus femoris (Binder-Macleod *et al.* 1998; Lieber and Kelly 1991).

2.3 Quadriceps muscle architecture

The majority of the muscle fibres within the quadriceps group are pennate and do not run parallel to the aponeurosis. The advantage of the fibres running at an angle to the aponeurosis is that a greater number of muscle fibres can be used to apply force to the tendon (Blazevich and Sharp 2005). Muscle architecture, which includes fibre pennation angle and fibre length, is a strong determinant of muscle force production capabilities (Lieber and Friden 2001). Large muscles, such as the quadriceps, have a large amount of sarcomeres set at an angle to the aponeurosis thus giving a greater physiological cross sectional area (PCSA) leading to greater force production (Lieber and Friden 2001).

Ultrasound techniques reveal that not only are there differences in architectural properties between the muscle groups but also that variation is evident along the length of the individual muscle (Blazevich *et al.* 2006). The authors suggested that these highlight the different roles and recruitment

patterns of the individual muscle fibres during a contraction. Their interpretation of these observations was that intravariability within muscle fibres is such that some sections of the muscle are for force generation whilst others are for force transfer (Blazevich *et al.* 2006). The force generating fibres would be more pennate for generating high forces and the force transfer section more parallel to the tendon for efficient transfer of forces through the tendon. This would have implications for the interpretation of muscle biopsy data in terms of the role of the fibres sampled and the muscles' previous activation pattern.

There have been many studies conducted to assess the effects of training on alterations in muscle architecture (Aagaard *et al.* 2001; Rutherford and Jones 1992) with equivocal findings. Rutherford *et al.* (1992) reported no changes in fibre angle following 3 months of strength training. This was despite a significant increase in MVC and cross sectional area. One reason for this finding suggested by the authors was that the ultrasound technique used was not sensitive enough to detect small changes in fibre pennation. A flaw in the study by Rutherford *et al.* (1992) was that they only measured the vastus lateralis and intermedius. Improvements in MVC may have come from changes within the rectus femoris and vastus medialis. Aagaard *et al.* (2001) conducted a similar length training study (14 weeks) and although they only measured the vastus lateralis they reported significant changes in pennation angle from 8.0° to 10.7°. A possible reason for this conflicting finding is that the range of exercises used in the two studies differed. Aagaard *et al.* (2001) utilised a range of leg exercises and also manipulated the intensities of the sessions, whilst Rutherford *et al* (1992) used only one exercise and one exercise intensity. Kawakami *et al.* (1992) showed a significant difference in

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fibre pennation between untrained and trained subjects. Muscle fibres of the strength trained subjects showed greater hypertrophy and this was significantly related to fibre pennation. Although it has been suggested that a significant increase in pennation angle may be detrimental for force production, the increase in PCSA would mean greater contractile elements applying force to the tendon (Fukunaga et al. 1997b). Fukunaga et al (1997a) reported fascicle length and angle (pennation) alterations during passive and static contractions of the quadriceps over a range of knee joint angles. Fascicle length was significantly shorter during the static contractions. Fascicle angle was only increased significantly during static contractions compared to passive when the knee angle was between 30° and 0° (0° = full extension). Also, the static contractions were only performed at 10% of the subject's MVC and architectural changes during contractions at higher force outputs have yet to be reported. However, Brancaccio et al. (2007) showed a significant increase in quadriceps thickness and pennation angle following a cycle ergometer test to exhaustion. An increase in pennation angle following an acute bout of exercise would lead to a reduction in the force produced by the muscle as a result of the greater angle in relation to the aponeurosis. Unfortunately no measures of muscle performance were measured before or after the exercise bout to report the effects of the change in pennation on muscle performance.

Alterations in tendon dynamics have been shown to influence force output from the muscle (Reeves et al. 2003). An increase in tendon stiffness has been reported following training, leading to an increase in force transmission (Magnusson et al. 2008; Narici et al. 2008; Reeves et al. 2003). Tendon

reflexes have been reported to change acutely depending on the preceding exercise (Rittweger *et al.* 2003). This alteration could have direct implications for the measurement of rate of torque development and rate of relaxation parameters before and after exercise.

It is clear that architectural properties such as muscle fibre pennation angle and length play a key role in the contraction characteristics of the muscle. Figure 2.2 demonstrates the relationship of fibre length and cross sectional area (CSA) of key muscles in the upper half of the lower limb. Clearly the CSA varies throughout the vasti group with similar fibre length. The greater fibre length of the semitendinosus (ST), with a lower CSA, reflects its role as a low force, high excursion muscle in comparison to the high force, low excursion of the vastus muscles.

Figure 2.2 Fibre length and cross sectional area of muscles of the lower limb (VL –Vastus Lateralis; VI – Vastus Intermedius; VM – Vastus Medialis; RF – Rectus Femoris; Biceps Femoris; ST – Semitendinosus). (Figure adapted from Lieber *et al.* (2001)

Burkholder *et al.* (1994) examined the relationship between muscle architecture and muscle fibre type and surmised that architectural properties did not relate directly to fibre type properties. Muscles with architectural properties for high velocity movements (long muscle fibres) did not necessarily contain high percentages of fast contracting muscle fibres. The muscle fibre properties were more related to activity levels. It is important when assessing muscular performance that the muscles' length/tension relationship is considered.

One of the muscles sampled most commonly in the quadriceps group is the vastus lateralis. Lexell *et al.* (1985) studied the variation in muscle fibre type composition of this muscle in both young (19-35yrs) and old (68-86yrs) subjects. They reported variation in fibre type within the same fibre and there was also a larger difference between superficial and deep muscles in the muscle of the young muscle but not the old (Sjostrom *et al.* 1986).

Research has highlighted variability in muscle morphology across the quadriceps group (Lieber and Friden 2001; Willan *et al.* 1990; Willan *et al.* 2002). Knight *et al.* (2005) demonstrated that the superficial motor units of the vastus lateralis were larger than those of the deeper muscles. They reported 114 motor units in total. These findings could be a critical factor when formulating conclusions on the muscle characteristics of the quadriceps. Variation between subjects in the muscle fibre type orientation could have implications for the response to electrical stimulation when applied directly over the muscle, where it has been suggested that superficial fibres are more easily recruited (Feiereisen *et al.* 1997).

Muscle fibre type percentages of the vastus lateralis have been reported to range from 40-50%, 20-40% and 15-22% in type I, IIA and IIB respectively (Saltin *et al.* 1977; Simoneau and Bouchard 1989; Staron *et al.* 2000). In endurance runners 68%, 25% and 3.5% have been reported for type I, IIA and IIX respectively (Saltin *et al.* 1977; Tesch and Karlsson 1985). Tesch *et al.* (1985) reported no difference in the mean fibre area of Type I muscle fibres between trained and sedentary (approximately 57%) but a significant difference in mean Type II area.

There is evidence for increased capillarisation within the muscle group following training. Andersen *et al.* (1977) reported a capillary density within the vastus lateralis of 329 (+/- 11) cap.mm^{-2} in healthy males before the commencement of an 8-week endurance training intervention. Following training, capillary density increased to 395 (+/- 16) cap.mm^{-2}.

2.4 Conclusion

This Chapter reports the main structure and characteristics of the quadriceps femoris, the main muscle of interest within this thesis. Its key role in human locomotion, from walking through to high intensity athletic performance, demonstrates the importance of understanding its function and the effects various interventions have on its performance. The Chapter emphasises the importance of muscle fibre type and muscle architecture when evaluating muscle contractile characteristics.

3 Development of an electrical stimulation protocol

3.0 Summary

As stated in Chapter one, electrical stimulation measures have been extensively used in clinical studies for the assessment of muscle function. Its validity, sensitivity and reliability for evaluating muscle performance have not been established in healthy, sporting populations. This Chapter outlines the development of the electrical stimulation protocol used throughout the thesis for the assessment of skeletal muscle performance. Fatigue resistance, rate of torque development and rate of relaxation will be assessed.

3.1 Introduction

It is imperative that any method utilised to measure an outcome is both precise and reproducible (Vincent 2005). A range of measures for assessing exercise intensity were discussed in Chapter one, however the focus of this thesis is on the measure of muscle performance. The function of a muscle or group of muscles is influenced by a number of factors such as fibre type composition, length of the muscle, force/velocity relationship and also by motivation of the subject, especially if a maximal effort is required. In any type of testing requiring effort from the subject, motivation can affect the outcome. Electrical stimulation techniques can assess muscle function independently of central factors, therefore allowing the assessment of alterations in performance at the muscle level (Stokes *et al.* 1988).

Many protocols that have utilised stimulation techniques have monitored the muscle at a fixed muscle length (Skof and Strojnik 2006b; Skurvydas *et al.* 2007; Vanderthommen and Duchateau 2007). Kooistra *et al.* (2005)

demonstrated that, at differing knee angles, endurance time was reduced at greater knee angles. Muscle perfusion and activation levels were not responsible for the reduction, demonstrating that the mechanics of the muscle play a key role in its function, so it is vital that this is controlled.

Crucially, electrical stimulation can assess the muscle using the same recruitment patterns, however it is imperative that the protocol utilised is comfortable for the subject, to ensure compliance and the ability to repeat the test. With a range of stimulation parameters being utilised in the research literature it is not evident which protocol, if any, offers the most effective, reliable and comfortable assessment of muscle characteristics. The use of electrical stimulation techniques have been utilised to assess *in vivo* muscle contractile characteristics, such as fatigue resistance and force and relaxation profiles, for many years in clinical (Edwards *et al.* 1977a; 1977b; Hamada *et al.* 2004; Scott *et al.* 1985; Scott *et al.* 1986; Sinacore *et al.* 1994) and recently in more healthy populations (Bentley *et al.* 2000; Skurvydas *et al.* 2002; Theurel and Lepers 2008). It has proved to be a reliable measure when stimulating at forces equating to 20-30% of an individual's maximal voluntary isometric contraction (MVIC) with a coefficient of variation ranging from 3-10% (Hanchard *et al.* 1998) and interclass coefficients of between 0.94-0.98 (Place *et al.* 2007). Various stimulation methods have been utilised in transcutaneous electrical stimulation, with a combination of pulse duration and pulse train durations used (Binder-Macleod *et al.* 1995; 2001; Scott *et al.* 1986). Pulse durations have ranged from between 50 µs to 1ms (Edwards *et al.* 1977a; Garland *et al.* 2003; Shields 1995) with pulse train duration from 250ms to 330ms (Edwards *et al.* 1977b; Scott *et al.* 1986; Scott *et al.* 2006).

Shorter pulse durations (i.e. 50 µs) require greater current amplitude (mA) to produce a maximal response. Likewise, longer pulse durations (~300µs) require less current for the same response and have also demonstrated a greater central contribution (Collins 2007). However, due to the shape of the current/duration relationship, pulse durations greater than 300 µs require the same current to produce maximal activation. Manipulation of the pulse train duration also affects the response. The manipulation of this parameter, along with the recovery time in between stimulations, will have a dramatic effect on the demand placed on the muscle.

As with the range of stimulation protocol, stimulation frequencies have also varied between studies, ranging from 1Hz to 100Hz. Stimulation at high frequencies (>40Hz) are often painful for participants. Frequencies during voluntary activation are rarely greater than 50Hz (Jones 1996) and therefore stimulating at frequencies greater than this does not seem warranted. Utilising the shape of the force frequency curve it should be possible to limit the discomfort of the testing protocol by limiting the maximum stimulation frequency to the area of the curve where an increase in frequency has a minimal effect on force output. Although work has been done to assess the differences in force response across a range of stimulation frequencies and pulse durations following fatigue (Chou and Binder-Macleod 2007), no work has reported the fatigue response to commonly used pulse train durations of 250 and 330ms. It is not clear whether there is any significant difference between absolute and normalised muscle contractile characteristics collected using pulse train durations of 250ms and 330ms. To address this, this study has these aims/questions:

1. Is there a difference in muscle contractile characteristics when using stimulation train durations of 250 and 330ms? Is there a benefit in using longer periods of stimulation for the assessment of muscle contractile characteristics?

2. Is there a significant difference in force output between force frequencies at 40 and 50Hz?

3.2 Assessment of muscle function

3.2.1 Equipment and subject set up

All muscle function tests throughout this thesis were conducted on a specially designed strength-testing chair. The design and validity of the testing set up has been reported elsewhere (Hyde *et al.* 1983) however, a description of its function is warranted.

The chair itself is fully adjustable for the subject, with the back of the chair having the ability to move forward or back to adjust for different thigh lengths. It also has a tilt function to optimise the level of back support. Figure 3.1 illustrates the position of the subject on the chair.

The device for recording torque outputs is connected to the front of the chair via steel rods. The components of the measuring device and the position of the subject on the chair are displayed in Figure 3.0.

Electrodes

Strain gauges

Lever arm

A

Amplifier and pulse
parameter settings

Stimulator

Analogue to digital
convertor

B
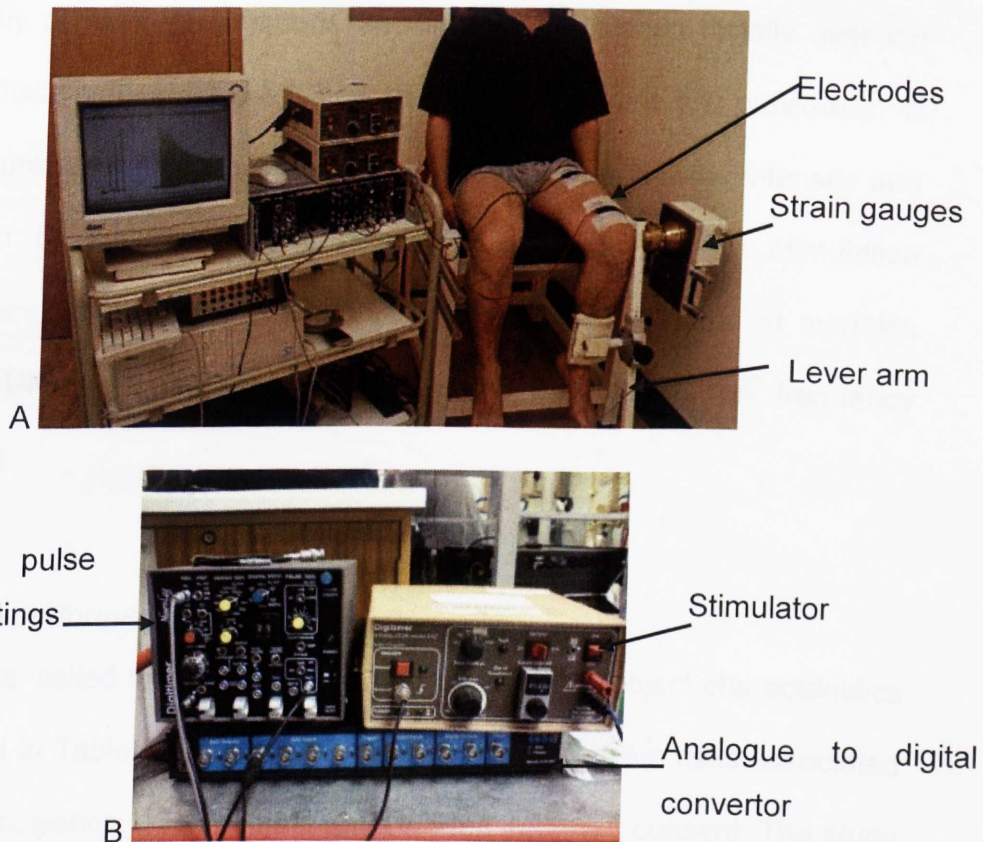
Figure 3.0 Layout of strength measuring device and subject position.

The subject/chair interface is via a padded plate attached to the lever arm (Figure 3.0). This is also fully adjustable so that the bottom of the plate is in line with the subject's ankle joint line. Four strain gauges within the measuring device measured the force outputs. The signal was first amplified (Digitimer Neurolog NL107, UK) and digitised (Cambridge Electronic Design micro 1401, UK). Outputs were displayed and stored using an analysis package (Spike 2 version 5, Cambridge Electronic Design, UK) for subsequent analysis. The measuring device was calibrated by sequentially applying known weights (10kg stages) to the load arm in the plane in which testing would be conducted. The linear results were then used to calculate torque output. Electrical stimulations were delivered on to the quadriceps of the subjects via two carbon-rubber electrodes, one placed proximally over the

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muscle belly of the rectus femoris and the second placed distally over the vastus medialis as described by Scott *et al.* (1986) (Figure 3.1) previously. A constant current stimulator was used to deliver and control the intensity and pulse width of the stimulations (Digitimer DS7, UK). The stimulation characteristics (pulse width, frequency) were set from a range of modules (Neurolog, UK); Pulse duration (NL401, UK) and stimulation frequency (NL301, UK).

3.2.2 Muscle performance

Eight subjects visited the laboratory on four occasions (subject characteristics are displayed in Table 3.0). After being fully informed of the risks associated with their participation, each subject gave written informed consent. The study was approved by the local University Ethics Committee (UREC 040083) and carried out according to the Declaration of Helsinki (Loff and Black 2000). Subjects sat in an upright position with the knee and hip held at 90° (Figure 3.1). Shoulder and lap straps restricted upper body movement in order to isolate muscle action. The axis of the lever arm attached to the force-measuring device was aligned with the knee flexion-extension axis whilst the subjects' tibiae were placed behind a plate, connected to the lever arm, 3cm above the lateral malleolus. The chair was fully adjustable for the individual and the position on the chair was recorded for subsequent testing.

3.2.3 Maximal voluntary isometric contractions

Three maximal voluntary isometric contractions (MVIC) were carried out on each visit (Theurel and Lepers 2008). Subjects were instructed to push against the plate attached to the lever arm as hard and as fast as they could.

Each effort was separated by one minute. If the final MVIC effort was the highest, further contractions were made until there was no more improvement. MVICs were required to set the appropriate electrical stimulus amplitude.

3.2.4 Electrical stimulations

Electrical stimuli were set at an appropriate intensity to elicit force outputs of 20% of the subject's MVIC. Force frequency data were collected using five pulse trains at 1, 10, 20, 40, 50 and 100Hz followed by a fatigue protocol conducted at 40Hz for 180s. Of the four sessions, two were conducted using a pulse train duration of 250ms and a further two at 330ms to allow comparison of the lengths of stimulation time. A pulse width of 300µs was used for all testing as this time period allows maximum excitation with minimum increases in stimulation intensity (Benton *et al.* 1981) . The sessions were completed in a randomised order. Absolute and normalised force frequency and fatigue indexes from each of the stimulation durations were reported. MVIC and electrically stimulated force outputs were collected and analysed using Spike data analysis software (Spike 2, Cambridge Electronic Design, UK).

3.2.5 Data analysis

The amount of fatigue was calculated as a percentage of the initial torque lost over the 180s period using the following equation:

$$\text{Fatigue Index (FI)} = \frac{\text{Initial Force} - \text{Final Force}}{\text{Initial Force}} \times 100$$

Eq. 1

(Scott *et al.* 1986)

A low fatigue index was thus indicative of greater fatigue resistance and a high fatigue index indicative of greater fatiguability. For comparison of the 250ms and 330ms pulse train duration protocols, the force outputs throughout the fatigue test were also normalised. This was done by reporting the force output for each of the 180 stimulations as a ratio of the maximum force output using the following equation:

$$\text{Normalised Fatigue Index} = \frac{\text{Force Output}}{\text{Maximum Force Output}}$$

Eq. 2

(Scott *et al.* 1986)

Force frequency data was expressed as absolute torque and also normalised using equation 3.

$$\text{Normalised force frequency} = \frac{\text{Force frequency output}}{\text{Maximum Force frequency output}}$$

Eq. 3

(Scott *et al.* 1986)

3.2.6 Statistical analysis

Descriptive statistics include mean +/- standard deviation for all measured variables. Data was examined for normality using a Kolmogorov-Smirnoff test.

Student t-tests were utilised to assess any differences between absolute and normalised force frequency and fatigue indexes from the two stimulation protocols. Student t-tests were also performed to assess any differences between 40 and 50Hz force frequency outputs. Statistical significance was accepted at $p < 0.05$.

3.3 Results

Table 3.0 illustrates the subject characteristics participating in the study.

Table 3.0 Subject characteristics (mean (SD)).

Age	Height (m)	Weight (kg)	MVC (Nm)
23.6 (5.7)	1.72 (0.13)	72.3 (16)	501.3 (116.4)

There was a significant difference between the absolute force frequency outputs from the 250ms and 330ms protocols ($p = 0.02$). However, when the data was normalised to the 40Hz force output (FFR/40Hz) there was no difference between the stimulation protocols ($p > 0.05$, Figure 3.1).
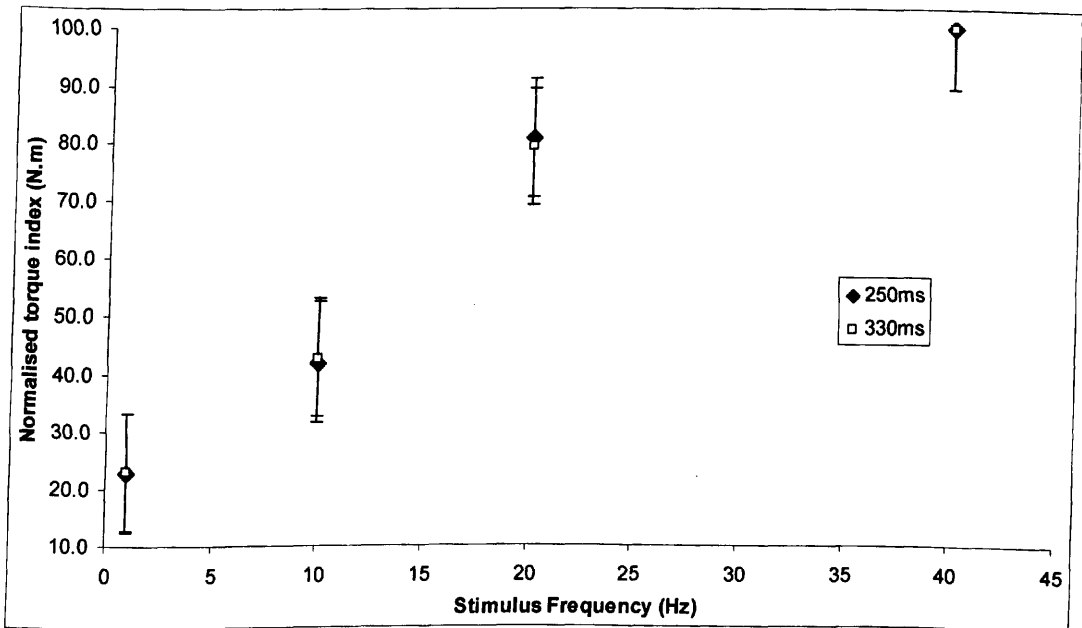
Figure 3.1 Mean (SD) normalised force frequency response from the 250ms (closed symbol) and 330ms (open symbol) trials (p > 0.05)

There was no significant difference in the amount of fatigue elicited by the fatigue protocol between the two stimulation periods (p = 0.51). Although the force output was higher throughout the fatigue test when utilising the 330ms stimulation period (Figure 3.2A), when the force outputs were normalised to the highest force output (usually within the first five pulses) there was no difference in the force output curves (Figure 3.2B).
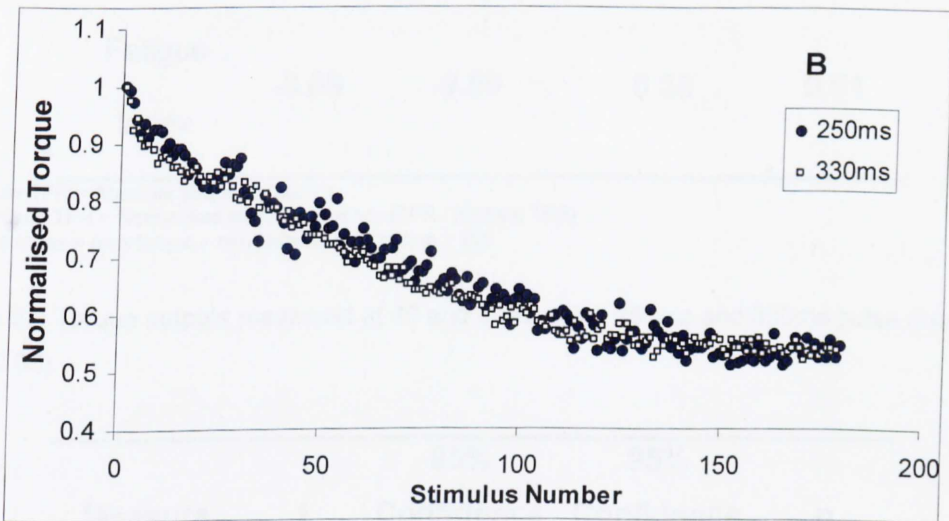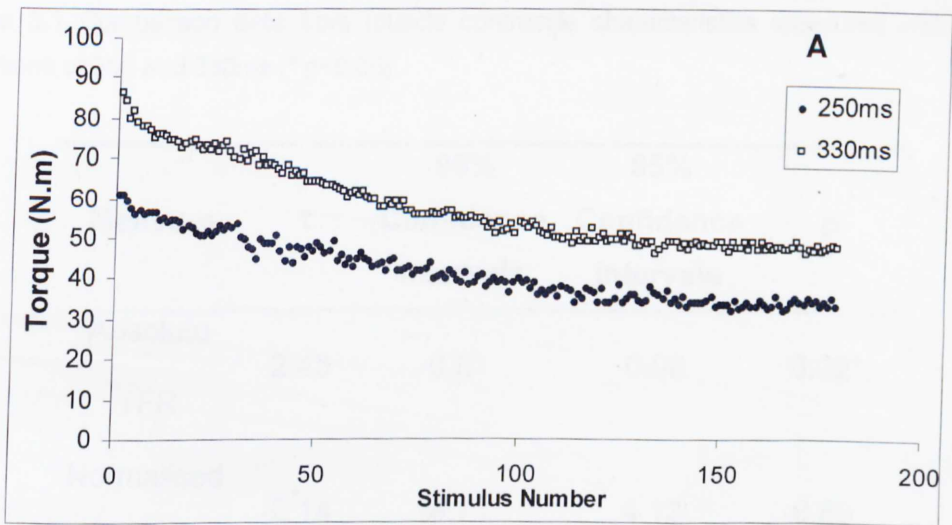
Figure 3.2 Typical trace from one subject for **A** raw force outputs and **B** normalised force outputs throughout the 3 minute fatigue protocol.

In addition, there was no significant increase in force output at frequencies of 40 and 50Hz (40-50Hz difference) at either of the pulse durations (p = 0.07 and p = 0.24 for 250ms and 330ms respectively (Table 3.2). Table 3.1 summarises the comparative data from the two stimulation periods.

Table 3.1 Comparison data from muscle contractile characteristics measured with pulse durations of 250 and 330ms (* p<0.05).

Measure	t	95% Confidence intervals	95% Confidence intervals	p
Absolute TFR	2.43	0.01	0.08	0.02*
Normalised TFR	-0.14	-4.71	4.12	0.89
Fatigue Index	-0.69	-9.86	5.38	0.51

Absolute TFR = Absolute torque frequency
Normalised TFR = Normalised torque frequency (TFR / Highest TFR)
Fatigue Index = max torque – min torque / max torque x 100

Table 3.2 Torque outputs measured at 40 and 50Hz using 250ms and 330ms pulse durations (* p<0.05).

Measure	t	95% Confidence intervals	95% Confidence intervals	p
40-50Hz diff (250ms)	-2.20	-0.03	0.002	0.07
40-50Hz diff (330ms)	-1.29	-0.07	0.02	0.24

40-50Hz diff = Difference in torque outputs between 40 and 50Hz

3.4 Discussion

There was no significant difference in the amount of fatigue elicited by stimulation protocols utilising different pulse train durations. Absolute torque outputs between the protocols differed but this difference was not significant when normalised. The torque output at 50Hz was not significantly greater

than that produced by 40Hz stimulation during either stimulation pulse train durations. Stimulations at 40 Hz were also deemed more comfortable than at 50 Hz and therefore justified only stimulating up to 40 Hz in future trials.

The findings from this study demonstrate that the absolute torque outputs are different when stimulating at the same frequency but with varying train duration. This finding is perhaps not surprising when considering the results from studies utilising stimulation trains consisting of a range of pulse number (Binder-Macleod and Barker 1991; Maladen et al. 2007). Maladen et al. (2007) assessed pulse trains consisting of 6, 13 and 25 pulses. Although they looked at dynamic muscle actions (compared to the isometric actions in this current study) they found that an increase in the number of pulses caused greater knee extension. The longer stimulation period of 330ms utilised in this current study meant that there was a greater number of pulses per train compared to the shorter 250ms protocol. This is reflected in the higher absolute torque outputs reported. This supports the work of Maladen et al (2007) demonstrating that the increase in the number of pulses increases the torque in isometric as well as dynamic muscular actions.

The timings utilised in this study are common stimulation periods used in the scientific literature (Binder-Macleod and Snyder-Mackler 1993; Burke et al. 1973; Fuglevand et al. 1999; Scott et al. 1990; Scott et al. 2006). However, although there was a difference in absolute torque outputs, when the torque frequency data was normalised (Binder-Macleod and Barker 1991; Scott et al. 1985; Snyder-Mackler et al. 1993), there was no significant difference between the torque outputs recorded using stimulation periods of 250ms or

330ms. To our knowledge this is the first study to assess the torque outputs between these two commonly used stimulation protocols. This is important when comparing findings from previous studies using these stimulation protocols as the normalised data is comparable.

The amount of fatigue induced by the two different stimulation periods, as measured during the fatigue protocol, was not significantly different. Binder-Macleod et al. (1995) assessed force frequency and fatigue characteristics in the quadriceps when stimulated at different frequencies and varying percentages of the subjects' MVIC. They reported no difference between normalised force frequency when the muscle was stimulated at 20% and 50% MVIC. This supports the findings from this current study comparing the different pulse durations. The absolute torque outputs were higher in the 330ms trial but, when normalised, there was no difference in the torque frequency data (Figure 3.2). However, Binder-Macleod et al. (1995) also reported that the amount of force reduction following a fatiguing protocol was greater at an intensity of 50% MVIC compared to 20% MVIC. We envisaged that the greater torque outputs elicited by the longer pulse train durations in this current study would also have led to greater fatigue such as that demonstrated by Binder-Macleod et al. (1995). However, a possible reason for there being no difference between the protocols in this study is because the difference in the stimulation periods was not wide enough to cause a greater disruption in the force capabilities of the stimulated muscle. Another possible reason is that a greater number of motor units will be recruited with longer pulse durations and therefore the amount of force generated per muscle fibre will be less (Kesar and Binder-Macleod 2006).

Some key developments are reported here to aid the development of subsequent experiments. Firstly there was no significant difference between the torque generated at 40 and 50Hz (Table 3.2). The torque outputs generated by stimulation at 40Hz were over 95% of that produced by 50Hz stimulation. This is in agreement with findings by Shields and Chang (1997) and Binder-Macleod et al. (1991). Binder-Macleod et al. (1991) reported that force outputs at a stimulus of 40Hz was greater than 90% than that of the force output at 100Hz. Binder-Macleod et al (1991) utilised a pulse duration of 350µs and stimulation period of 300ms. The findings from this current study, along with those from previous work, meant that subsequent testing of FFR data at 50Hz was no longer warranted and would thus aid the more rapid collection of data in future studies within the thesis. This was also supported by the finding that the stimulations at 50Hz were reported as being uncomfortable by the majority of the subjects. Utilising stimulation frequencies up to 40Hz also reflects the natural firing frequencies, reported during voluntary muscle actions, of between 10 and 30Hz reported during maximal voluntary contractions (Bellemare et al. 1983). The torque frequency ratios reported in this current work are also in line with those reported in published studies (Edwards et al. 1977b; Hyde et al. 1983).

Following the findings of this current study, the 250ms protocol was selected for use in subsequent testing throughout this thesis as there was no significant difference in the amount of fatigue recorded between the different stimulation times and the normalised torque frequencies. Utilising this protocol also kept it in line with those used by a collaborative institute at the University of East London. The finding that there was no significant difference between the

torque outputs elicited by 40 and 50Hz, at either of the stimulation durations meant that the testing protocol could be modified for subsequent studies throughout this thesis.

3.5 Development of analysis techniques and their reliability

3.5.1 Introduction

The previous section established the electrical stimulation protocol to be utilised throughout this thesis for the assessment of muscle performance. However, its reliability as a measure of muscle performance has yet to be reported. Burke *et al.* (1973) developed a protocol for assessment of muscle contractile characteristics in cats. This model has been utilised and modified in a number of studies for assessment of muscle performance (Binder-Macleod and Snyder-Mackler 1993; Davies and White 1982; Scott *et al.* 1986). The fatigue index as measured in these studies has shown to be a useful indicator of training status of the muscle (Garland *et al.* 2004; Scott *et al.* 1986). Although the findings are equivocal, studies have suggested that the shape of the fatigue curve could be indicative of muscle fibre type composition (Snyder-Mackler 1993). The findings to date suggest that the shape of the fatigue curve may be a genetic trait (Scott *et al.* 1986) and is only altered by prolonged periods of training (Garland *et al.* 2004) or indeed detraining (Snyder-Mackler *et al.* 1993). If the fatigue curve is also reflective of fibre type composition it could possibly offer an insight into muscle recruitment patterns during various forms of exercise and the effects of exercise intensity. The fatigue protocol has been utilised to measure muscle function compared to the $\dot{V}O_2$ slow component (Garland *et al.* 2004), to compare the fatigue profile of different muscle types (Bigland-Ritchie *et al.*

1986) and after surgery (Snyder-Mackler *et al.* 1993). Following ACL surgery, the involved quadriceps was more fatigue resistant than the uninvolved. This was demonstrated in a shallower drop-off in torque over the first minute of the fatigue protocol and a less drop-off in torque overall (Figure 3.3). Snyder-Mackler *et al.* (1993) postulated that this was due to either a greater recruitment of more fatigue resistant muscle fibres or a greater atrophy of the Type II muscle fibres in the involved muscle group. If the fatigue curve indicates fibre type availability it could offer an insight into muscle recruitment patterns following various forms of exercise and exercise intensity.
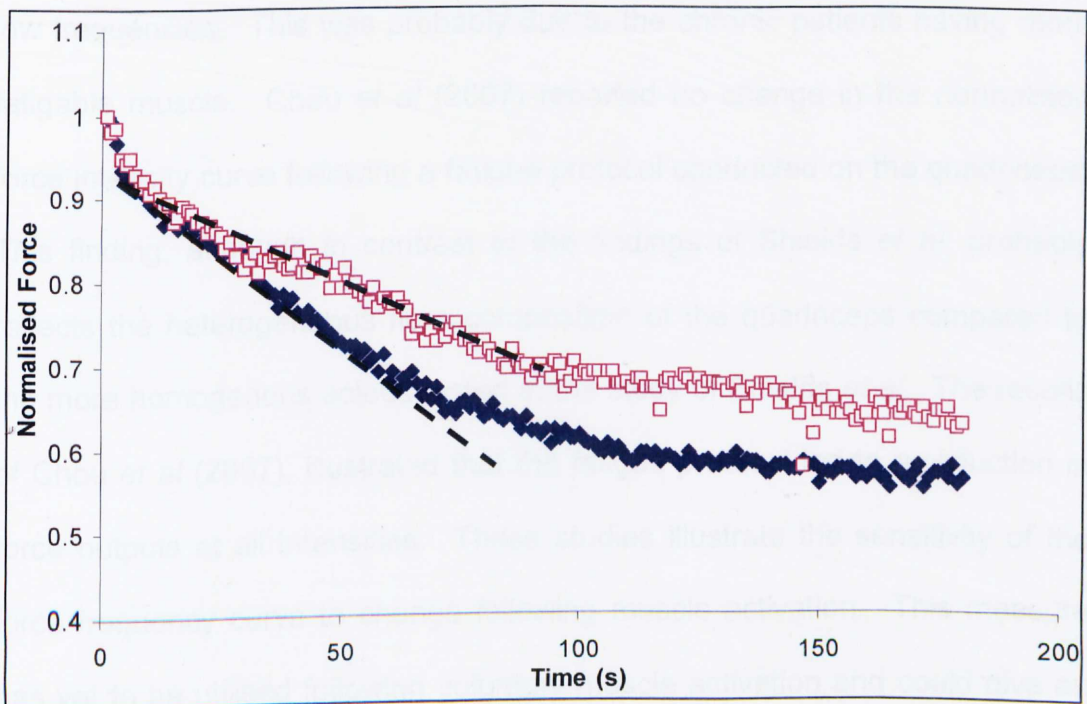
Figure 3.3 Example of greater fatigue resistance (open symbols) during a fatigue protocol similar to that utilised by Snyder-Mackler *et al* (1993). The broken lines highlight the reduced rate of torque decline over the first 60s of the test in the subject displaying greater fatigue resistance (unpublished data M.Morris).

Torque frequency profiles (TFR), similar to those displayed in Figure 3.1, have been used in the clinical setting to prescribe optimum stimulation patterns for functional electrical stimulation (Binder-Macleod *et al.* 1998). Measures of

FFR have been reported following electrical stimulation protocols and various exercise bouts in an attempt to quantify the effects of the intervention on skeletal muscle. Shields and Chang (1997) measured the frequency curve in paralysed soleus muscle before, during and after a fatiguing protocol in patients with acute and chronic paralysis. In the chronic patients the effects of the protocol caused the force frequency curve to shift to the right. This was evident even when the force outputs were normalised to the highest frequency. A higher stimulus frequency was therefore required to attain the same relative force output, due to a greater reduction in forces produced at low frequencies. This was probably due to the chronic patients having more fatigable muscle. Chou et al (2007) reported no change in the normalised force intensity curve following a fatigue protocol conducted on the quadriceps. This finding, although in contrast to the findings of Shields et al, probably reflects the heterogeneous fibre composition of the quadriceps compared to the more homogenous soleus tested in the study of Shields et al. The results of Chou et al (2007), illustrated that the fatigue protocol led to a reduction in force outputs at all intensities. These studies illustrate the sensitivity of the force frequency curve to change following muscle activation. This measure has yet to be utilised following voluntary muscle activation and could give an interesting insight into the changing muscle force dynamics at low and high frequencies, following exercise of varying intensity.

Rate of torque development (RTD) and rate of relaxation (RR) affect both fatigue and torque frequency response from the stimulated muscle. These measures are indicative of the acto-myosin complex. Calcium ion transients and myosin-ATPase play a key role in these measures and can give a useful

indication of the changing dynamics of the stimulated muscle. Different responses have been reported following exercise interventions, with muscle fibre type being suggested to influence the extent of alteration in RTD, RR or both (Hamada *et al.* 2003). Although a commonly used measure, very few studies have reported the reliability of the calculation of RTD and RR.

Along with direct measures of skeletal muscle, a number of studies have attempted to quantify central contributions to reduced muscle force output. A number of protocols have been used to assess central fatigue following exercise (Babault *et al.* 2006; Millet and Lepers 2004; Place *et al.* 2004; Taylor and Gandevia 2008). Place *et al* (2004) used the force frequency/MVC ratio as an indirect indicator of central fatigue. They demonstrated a significant reduction in the ratio following prolonged exercise. However this data was only collected on a small cohort of subjects. Further work is required in larger samples and following exercise bouts of various intensities.

Various *in vivo* techniques have been utilised to investigate muscle contractile characteristics such as, fatigue resistance, RTD and RR. However, very few have reported the reliability of these measures when stimulating the muscle directly. The aim of this section is to:

Examine the reliability of the data collection and analysis techniques for the assessment of muscle contractile characteristics, central to the work described later in the thesis.

3.6 Methods

Eleven healthy recreationally active males, mean age 26.1 ±3.7yrs, weight 78.1 ± 9.7kg and height 1.79 ± 0.05m participated in the study. After being fully informed of the risks associated with their participation, each subject gave written informed consent. The study was approved by the local University Ethics Committee and carried out according to the Declaration of Helsinki (2000).

All subjects were fully habituated to the testing procedures before participating in the study. Following habituation, subjects attended assessments on two occasions, each separated by one week. During each session muscle contractile characteristics were collected as described in Section 3.2.

3.6.1 Data analysis

3.6.2 Rate of torque development and rate of relaxation

Muscle contractile characteristics were analysed on resting muscle (baseline) and following the fatigue protocol described previously (study 3.2).

Rate of torque development and relaxation were calculated on torque outputs at the beginning and end of the fatigue test. Rate of torque and relaxation development were calculated from dF/dt and $-dF/dt$ (Gordon *et al.* 1990). This entailed calculating rate of torque development (RTD) from the slope of the force trace from baseline to peak torque following the electrical stimulus (Figure 3.4). Relaxation parameters were measured as rate of relaxation, calculated from the slope of the torque trace from peak torque back to baseline (RR) and rate of half relaxation, calculated from peak torque to half peak torque (RR ½) (Figure 3.4). Change in rate of torque development and relaxation were also assessed. The change in rate of torque development (% RTD) was calculated as the % change in rate of torque development from the

beginning to the end of the fatigue test.   Calculation of the % change in rate of relaxation (% RR) and the % change in RR $_{1/2}$ (%RR $_{1/2}$) was the same as that used for % RTD.
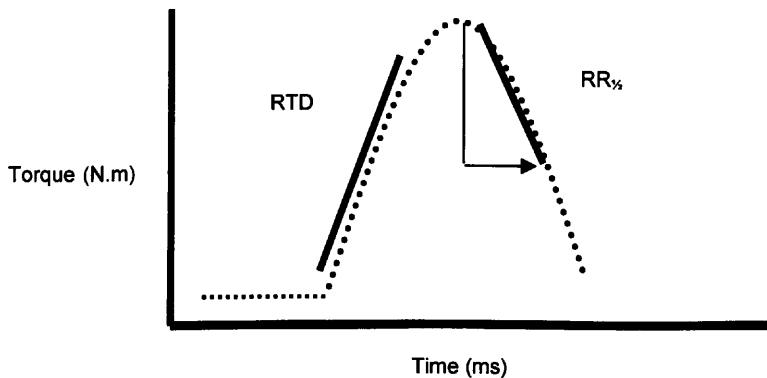
Figure 3.4 Areas of the torque response utilised for the calculation of rate of torque development (RTD) and rate of relaxation (RR$_{1/2}$).

3.6.3 Fatigue index (FI %)

The fatigue index was calculated from equation 1 in the previous study. Analysis of the slope of the fatigue curves was also assessed. In addition to the first 60s of the fatigue protocol, as utilised by Snyder-Mackler *et al.* (1993), assessments were made of the first 10 and 20s slopes to assess their reliability. From curve estimation a linear fit was selected. Figure 3.5 displays the area of the fatigue curve measured for each slope analysis.
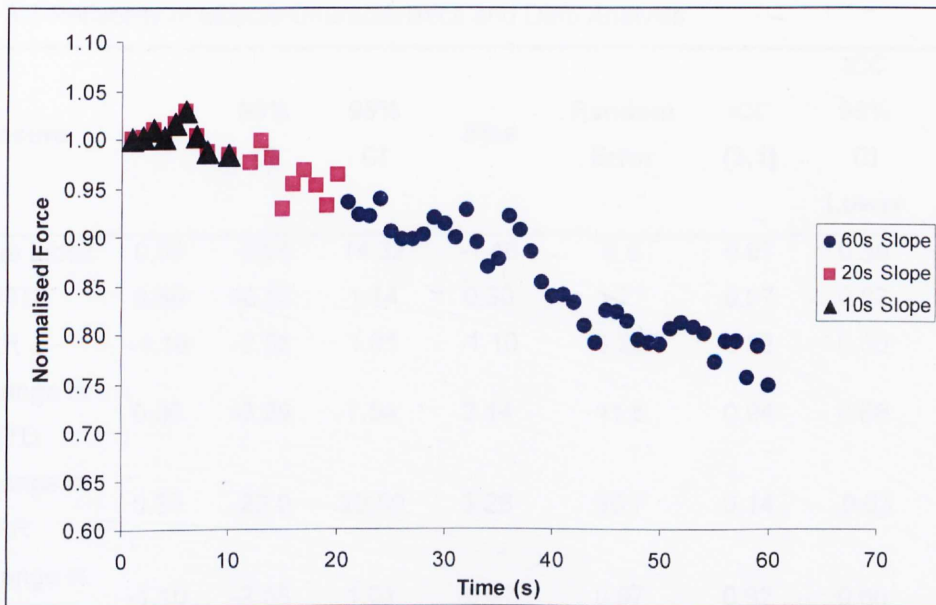
Figure 3.5 Areas of fatigue curve used for a typical slope analysis.

3.6.4 Torque frequency ratios and central fatigue (TFR and TFR/MVIC)

Each torque frequency was expressed as a ratio of the highest frequency utilised in the study (40Hz) as used in the studies of Scott *et al.*(1990) and Edwards *et al.* (1977b). Central fatigue was assessed by measuring the ratio of the highest stimulus frequency (40Hz) torque output to the maximal voluntary isometric contraction (TFR/MVIC).

3.6.5 Statistical analysis

Test-retest reliability was assessed on the muscle contractile measures using Student t-tests, intraclass correlation coefficient [3,1] and bias and random error analysis. Statistical significance was accepted at $p < 0.05$.

3.7 Results

All the methods used for the analysis of the electrical stimulation data were shown to be reproducible apart from the rate of relaxation, although the time to half relaxation (RR½) was reliable. Table 3.3 displays the reliability data collected on all of the analysis methods.

Table 3.3 Reliability of Muscle Characteristics and Data Analysis

Measure	t	95% CI	95% CI	Bias	Random Error	ICC [3,1]	ICC 95% CI Lower	ICC 95% CI Upper
Fatigue Index	0.56	-8.95	14.32	-1.46	6.9	0.97	0.88	0.99
RTD	0.89	-0.53	1.14	0.30	1.77	0.97	0.82	0.99
RR ½	-1.10	-2.58	1.01	-1.10	3.29	0.92	0.60	0.99
% Change in RTD	0.96	-3.29	7.54	2.14	11.5	0.94	0.68	0.95
% Change in RR	0.30	-23.0	29.50	3.26	55.7	0.14	-0.63	0.77
% Change in RR ½	-1.10	-2.58	1.01	-0.37	0.97	0.92	0.60	0.99
40:MVC	-2.10	-2.99	0.25	-1.40	3.40	0.98	0.93	0.99
FFR (% 40Hz)	0.48	-4.14	6.64	1.30	24.4	0.90	0.77	0.95
60s Slope	-1.05	-0.001	0.0005	-0.0004	0.003	0.86	0.57	0.96
20s Slope	1.00	-0.001	0.001	0.001	.0003	0.95	0.82	0.99
10s Slope	0.48	-0.014	0.009	-0.002	0.03	0.81	0.40	0.95

Fatigue Index = max torque – min torque / max torque x 100
Rate of torque development (RTD) = dF / dt
Rate of relaxation (RR½) = -dF peak / dt half peak
% Change in RTD = Initial RTD – Final RTD / Initial RTD x 100
% Change in RR½ = Initial RR½ - Final RR½ / Initial RR½ x 100
% Change in RR = Initial RR - Final RR / Initial RR x 100
40:MVC = 40Hz torque / MVC torque
60s Slope = Slope of first 60 s data points in the fatigue protocol
20s Slope = Slope of first 20 s data points in the fatigue protocol
10s Slope = Slope of first 10 s data points in the fatigue protocol

The normalised torque frequencies demonstrated good reliability between tests as demonstrated with an ICC of 0.90. Figure 3.6 displays the torque frequency outputs from test 1 and 2.
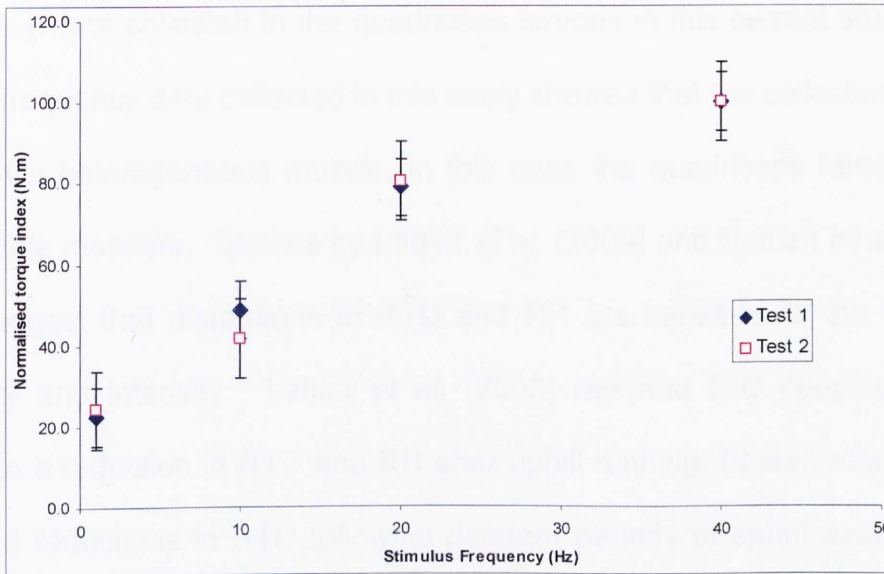
Figure 3.6 Test-retest data for the normalised torque frequencies (FFR / Max FFR)

3.8 Discussion

The findings from this section demonstrate that the electrical stimulation protocol and the analysis techniques to be used throughout the thesis are reliable as demonstrated by the ICC and t-test results in Table 3.3. There was no significant difference in fatigue resistance, slope of the fatigue curve, contractile characteristics (represented by rate of torque development and rate of relaxation) and torque frequencies between the two assessments.

There are few reports on the reliability of the analysis techniques in healthy populations (Place et al. 2007 and Hanchard et al. 1998). The fatigue index, rate of torque development (RTD), rate of half relaxation (RR½), change in RTD and RR½, the ratio of 40Hz torque output to MVC (40:MVC), normalised torque frequencies (Norm FFR) and the slope analysis were shown to be reliable measures. The percentage change in RR was the only measure to demonstrate poor reliability (ICC 0.14). The findings support those of Place et al. (2007) who reported similar ICC levels from muscle performance data collected using direct nerve stimulation. Although collected from tibialis anterior, findings from Hanchard et al. (1998) support the reliability of torque

frequency data collected in the quadriceps femoris in this current study. The torque frequency data collected in this study showed that the collection of FFR data on a heterogeneous muscle, in this case the quadriceps femoris, is a repeatable measure. Studies by Lattier *et al.* (2003) and Beelen *et al.* (1995) also suggest that disruptions in RTD and RR are sensitive to the exercise modality and intensity. Lattier *et al.* (2003) reported E-C coupling failure linked to a reduction in RTD and RR after uphill running. Beelen *et al.* (1995) reported reductions in RTD following different periods of sprint exercise. In this work, the longer sprint duration led to a greater reduction in RTD and other contractile characteristics, showing that measurements of RTD and RR are sensitive to the training history of the subject and also the intensity of the exercise. A reduction in RTD and RR parameters was reported in power-trained athletes only in a study by Garrandes *et al.* (2007), with presumably the power-trained group having a greater percentage of Type II muscle fibres in comparison to the endurance group. This current study demonstrates that the techniques are repeatable and can be used to improve our knowledge of the effects of exercise on skeletal muscle performance.

The findings support the use of the technique investigated in this chapter for the evaluation of possible peripheral and central factors involved with alterations in muscle contractile performance. The following Chapters will investigate muscle performance measures in relation to established markers of exercise performance to assess concurrent validity.

3.9 Conclusion

The work in the first section of this Chapter established the electrical stimulation protocol to be utilised throughout the thesis. With no significant difference reported between two commonly used stimulation protocols, the use of 250ms pulse train durations was selected to keep in line with those used by other collaborative institutes. Also, the finding that the torque output at 40Hz was not significantly different to that of 50Hz meant the protocol could be shortened for subsequent testing throughout the thesis.

The second section of this Chapter reported the reliability of the measures recorded from the electrical stimulation protocol. RTD, RR½, change in RTD and RR½, 40Hz:MVC ratio, Norm FFR and slope analysis all proved to be reliable measures of muscle performance.

4 Muscle fatigue characteristics: relationship to markers of endurance performance

4.0 Summary

Using the methodology developed in Chapter three, the aim of this Chapter was to assess the relationships between muscle contractile characteristics, collected using the electrical stimulation protocol, and other markers of endurance performance that reflect muscle performance including; $\dot{V}O_2$ max, lactate threshold, gross mechanical efficiency, peak power and the $\dot{V}O_2$ slow component. The results demonstrate a significant relationship between measures from the electrical stimulation protocol, the fatigue index, and lactate threshold. There was a trend for the percentage change in rate of torque development to relate to peak power. In those subjects tested, a trend was also evident between the fatigue index and the $\dot{V}O_2$ slow component. The muscle electrical stimulation protocol elicited measures that did reflect those of other established measures of muscle performance.

4.1 Introduction

To our knowledge the relationship of muscle characteristics to other determinants of exercise performance reflecting muscle performance, has not been fully explored utilising electrical stimulation techniques. Initial studies suggest that changes in muscle characteristics following exercise are sensitive to the intensity of the exercise session (Skof and Strojnik 2006a; Skof and Strojnik 2006b; Theurel and Lepers 2008). Muscle characteristics have also been shown to indicate aerobic performance during controlled leg extension exercise (Garland et al. 2004). As muscle fibre composition has demonstrated strong links with exercise performance and training (Coyle

1999; Horowitz *et al.* 1994) we would expect a similar relationship with muscle contractile characteristics collected from electrical stimulation protocols.

In this Chapter the contractile and fatigue characteristics of the quadriceps femoris muscle is examined in relation to established physiological markers of cycling endurance. We have included maximal oxygen uptake ($\dot{V}O_2$ max), gross mechanical efficiency (GME) and the lactate threshold (LT), as these are well validated determinants of endurance ability. Considering the findings in both animal (Gordon *et al.* 1990) and human studies (Garland *et al.* 2004) it was hypothesised that a greater fatigue resistance, as measured by a greater ability to maintain torque output during the electrical stimulation protocol, would correlate with a higher $\dot{V}O_2$ max, greater gross mechanical efficiency and a higher lactate threshold whilst a smaller reduction in rate of torque development (RTD) and relaxation rate (RR) would correlate with a higher $\dot{V}O_2$ max, greater gross mechanical efficiency and a higher lactate threshold.

4.2 Methods

Eleven healthy recreationally-active males (mean age 26.1 $\pm$ 4yrs, weight 78.1 $\pm$ 10 kg and height 1.79 $\pm$ 0.05 m) participated in the study. After being fully informed of the risks associated with their participation, each subject gave written informed consent. The study was approved by the local University Ethics Committee and carried out according to the Declaration of Helsinki (2000).

All subjects were fully habituated to the testing procedures before participating in the study. The habituation session allowed the subjects to experience the

sensation of electrical stimulation. If subjects were unfamiliar with the methods for collecting expired air this was also practised during this session. Following habituation, subjects attended assessments on three occasions, each separated by one week. In assessments 1 and 2, muscle performance data was collected. Assessment 3 consisted of the collection of $\dot{V}O_2$ max, lactate threshold and gross mechanical efficiency data. To minimise any diurnal effects all sessions were conducted at the same time of day (+/- 2 hrs). Prior to testing, subjects were asked to refrain from exhaustive exercise 48 hrs prior to the session, and to maintain their normal dietary habits, arriving at the laboratory in a euhydrated state. Six subjects returned to the laboratory on a fourth occasion and completed a $\dot{V}O_2$ slow component test.

4.2.1 Measurement of muscle performance

The method for the collection of muscle performance was as described in section 3.2. However, following the findings from Chapter 3, the electrical stimuli delivered to the muscle were at frequencies of 1, 10, 20 and 40Hz with a pulse duration of 250ms and pulse width at 300μs. Stimulation intensity ranged from 60-100mA.

4.2.2 Data analysis

Fatigue resistance was calculated as per equation 1 and normalised as per equation 2 (section 3.2.5). A low fatigue index was thus indicative of greater fatigue resistance and a high fatigue index indicative of lower fatigue resistance. The slope of the torque drop off after 10, 20 and 60 s, as described in 3.6.3, was also assessed.

Rate of torque development and relaxation were also calculated for torque outputs at the beginning and end of the fatigue test. The method utilised for the calculation of these parameters is described in 3.6.1. Baseline was established at the beginning of the testing session and was defined as a stable torque trace at rest for a 2-minute period before commencement of the electrical stimulation. Baseline remained stable throughout the testing session.

4.2.3 Measurement of peak oxygen consumption ($\dot{V}O_2$ max) and lactate threshold (LT)

Tests were conducted on an electromagnetically braked cycle ergometer (Lode, Gronigen, The Netherlands). Each subject was allowed to warm up for 5 minutes prior to following an incremental protocol similar to that utilised by Burnley et al. (2000). Subjects were instructed to maintain a cadence of 80 RPM throughout the test. Cycling commenced at a work rate of 70-100 W, and was increased by 20W every four minutes. At the end of each four-minute stage, a finger-prick blood sample was taken for lactate concentration analysis (Analox PGM-7, Hammersmith, London, UK). Once the blood lactate concentration had increased by 1 mmol^{-1} above the baseline level achieved during earlier work rates, the work rate was increased by 20 W every minute until volitional exhaustion. Pulmonary gas exchange was measured breath-by-breath using an automated metabolic analysis system (Oxycon Gamma, Jaeger, Hoechberg, Germany). Prior to each test, the gas analysers were calibrated with gases of known concentrations and flow volume was calibrated with a 3 L syringe (Hans Rudolph) according to manufacturer's specifications. Subjects wore a nose clip and breathed through a mouthpiece connected to a low-resistance volume transducer (Jaeger Triple V, Hoechberg, Germany).

Heart rate was recorded continuously throughout the testing protocol using short-range telemetry (Polar S810, Finland). Steady state $\dot{V}O_2$ was calculated as the average $\dot{V}O_2$ in the last 60 s of each four-minute stage, whilst $\dot{V}O_2$ max was recorded as the highest 30 s average before the termination of the test. Peak power output (PPO) was calculated as the sum of the final completed workload, plus the fraction of the partly completed workload performed before exhaustion (Moseley and Jeukendrup 2001). The lactate threshold was identified as the point at which blood lactate concentrations increased 1mmol^{-1} above baseline measures, as evident on plots of blood lactate versus work rate. This threshold was expressed as a percentage of $\dot{V}O_2$ max (LT-$\dot{V}O_2$).

4.2.4 Gross mechanical efficiency (GME)

For all work rates below the lactate threshold and below a respiratory exchange ratio (RER) of 1.00, GME was calculated as the ratio of actual work rate (kcal.min^{-1}) to the rate of calorific expenditure (kcal.min^{-1}) (Sidossis et al. 1992). Energy expenditure (kcal.min^{-1}) was calculated from $\dot{V}O_2$ and RER data (Lusk 1924).

4.2.5 Measurement of the $\dot{V}O_2$ slow component

Six subjects reported to the laboratory on a fourth occasion within a week of the $\dot{V}O_2$ max and lactate threshold session to complete a $\dot{V}O_2$ slow component test. The test was conducted on the same cycle ergometer. The session involved each subject performing a six-minute work bout at an intensity equating to 50% of the difference between the work rate at the lactate threshold and $\dot{V}O_2$ max (50% Δ) set from the previous maximal test. The test commenced with the subject sitting on the cycle ergometer and

baseline $\dot{V}O_2$ data was collected for two minutes. Following this, subjects started pedalling at the set intensity of 50% Δ which was maintained for six minutes. At the end of the six minute period the workload was reduced and the subjects allowed to recover. Gas exchange and heart rate data was collected continuously throughout the testing period as described in the $\dot{V}O_2$ max test. A blood lactate sample was taken immediately post exercise. The $\dot{V}O_2$ slow component was calculated from the difference between the six minute and three minute $\dot{V}O_2$ data (Garland *et al.* 2004).

4.2.6 Statistical analysis

Descriptive statistics include mean +/- sd for all variables measured and all data was examined for normality using a Kolmogorov-Smirnoff test. The relationship between variables was examined using a Pearson's correlation coefficient to assess whether there was a relationship between measured muscle characteristics and $\dot{V}O_2$ max, peak power, LT and GME. From curve estimation, both visual inspection and examination of R and R^2 values, linear regression was found to best describe relationships for all measures. Due to multiple tests, a Bonferroni adjustment was utilised and statistical significance was subsequently accepted at $p < 0.01$, so as to help avoid a Type I error.

4.3 Results

Mean and standard deviation data for $\dot{V}O_2$ max, LT, gross mechanical efficiency (GME), fatigue index, rate of torque development (RTD), rate of relaxation (RR½), % drop in torque development (ΔRTD) and % drop in relaxation rate (ΔRR½) are displayed in Table 4.0.

Table 4.0 Data for endurance performance determinants and muscle characteristic measures (mean +/- standard deviation.

	Mean	SD
$\dot{V}O_2$ max (L/min^{-1})	4.29	0.60
Peak Power (W)	336.4	42.9
LT-$\dot{V}O_2$ (% $\dot{V}O_2$)	61.3	6.8
GME (%)	18.4	1.0
Fatigue Index (%)	39.6	8.9
RTD (Nm/s)	302.2	104.9
RR $_{1/2}$ (Nm·s)	476.5	208.6
% Change in RTD	44.2	15.9
% Change in RR $_{1/2}$	36.3	17.1

$\dot{V}O_2$ = maximal oxygen uptake
Peak power = Highest power attained during the maximal cycle ergometer test.
LT- $\dot{V}O_2$ (% $\dot{V}O_2$) = Percentage of $\dot{V}O_2$ at which the lactate threshold occurs.
GME = Gross mechanical efficiency (actual work / energy expenditure)
Fatigue Index = max torque – min torque / max torque x 100
Rate of torque development (RTD) = dF / dt
Rate of relaxation (RR$_{1/2}$) = -dF peak / dt half peak
% Change in RTD = Initial RTD – Final RTD / Initial RTD x 100
% Change in RR$_{1/2}$ = Initial RR$_{1/2}$ - Final RR$_{1/2}$ / Initial RR$_{1/2}$ x 100

4.3.1 Relationship of muscle characteristics with measures of endurance performance

The fatigue index (FI) related significantly with LT, with subjects that displayed low fatigue resistance having a low LT and vice versa (Figure 4.0). In contrast there was no significant relationship between FI and $\dot{V}O_2$ max, GME and peak power. Table 4.1 displays the relationships of FI to endurance performance measures. There was also a significant relationship between FI and ΔRTD (r=0.79, p<0.01). Rate of torque development (RTD) and rate of relaxation (RR$_{1/2}$) did not relate to any endurance measures. This was also

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true for the ΔRTD and ΔRR$_{\frac{1}{2}}$. However, there was a trend between ΔRTD and

the endurance measures, in particular peak power and $\dot{V}O_2$ max (Table 4.2).

The slope of the fatigue curves after 10, 20 and 60s displayed no significant

relationships with any of the parameters.

Table 4.1 Relationships between the fatigue index and measures of endurance performance

Measure	Regression	r	p
LT-$\dot{V}O_2$	-0.48x + 80.0	-0.72	0.006*
Peak Power	-1.17x + 384.2	-0.23	0.25
GME	-0.07x + 21.0	-0.52	0.08
$\dot{V}O_2$ max	-0.02x +5.0	-0.40	0.11

(Following Bonferroni adjustment significance set at p<0.01)

Table 4.2 Relationships between %ΔRTD and measures of endurance performance

Measure	Regression	r	p
LT-$\dot{V}O_2$	-0.51x + 74.2	-0.55	0.05
Peak Power	3.2x +156.3	0.69	0.02
GME	-0.06x + 21.0	0.48	0.09
$\dot{V}O_2$ max	-0.04x + 6.1	0.56	0.04

(Following Bonferroni adjustment significance set at p<0.01)
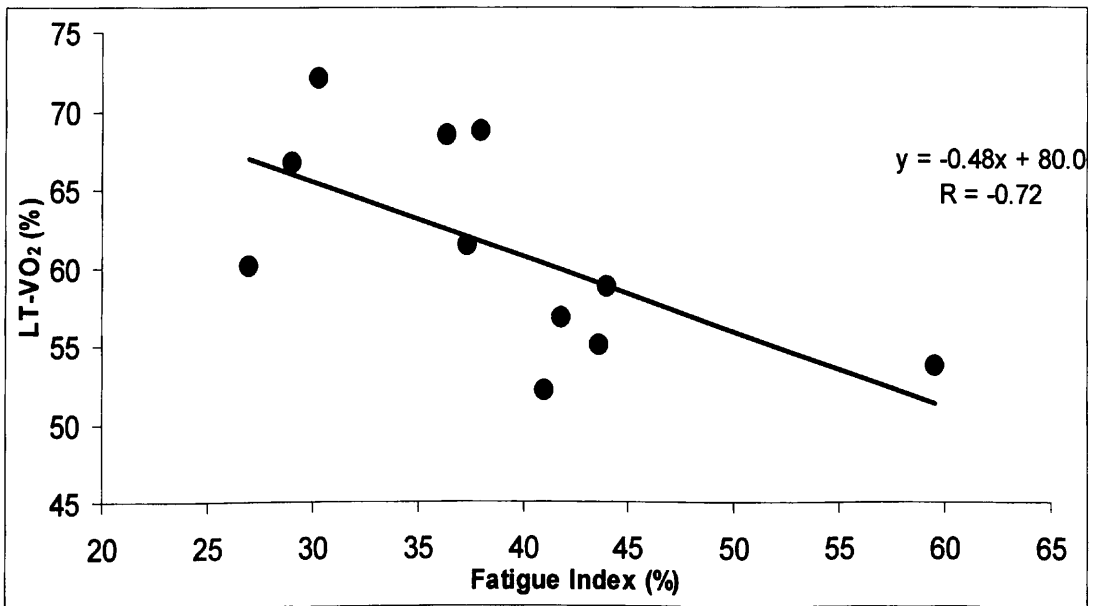
Figure 4.0 Relationship between LT- $\dot{V}O_2$ and the fatigue index

4.3.2 Relationship of fatigue resistance to the $\dot{V}O_2$ slow component

From the six subjects that completed the $\dot{V}O_2$ slow component test there was a trend for a relationship with the fatigue index but this did not reach significance (p0.17, R=-0.47) (Figure 4.1). Subjects displaying greater fatigue resistance had a greater $\dot{V}O_2$ slow component. Due to the small number of data sets this part of the study was not adequately powered and would require a larger sample size for any possible relationship to be established. Power analysis suggested that 17 subjects would be required.
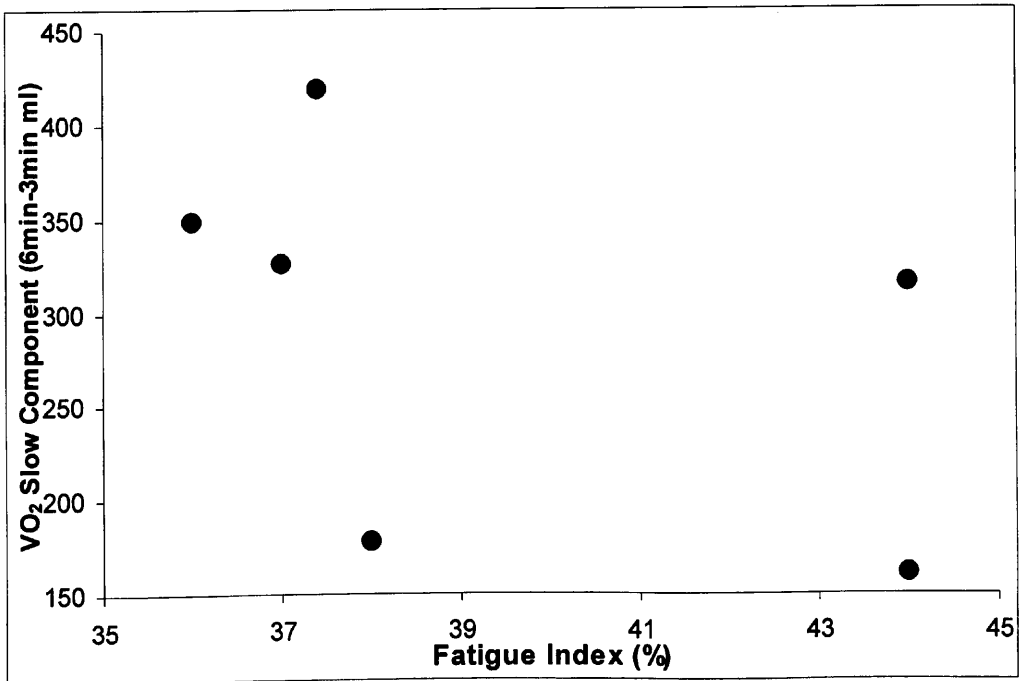
Figure 4.1 Relationship between the fatigue index and the $\dot{V}O_2$ slow component

4.4 Discussion

In agreement with previous observations that individuals who were endurance trained had less fatigable muscles (Garland et al. 2004), we observed that muscle contractile and fatigue characteristics were related to specific endurance performance determinants. Subjects displaying higher fatigue resistance during the muscle fatigue protocol demonstrated a higher lactate threshold in the cycle ergometer test. Interestingly, the fatigue index did not relate to the general marker of cardiovascular performance as measured by $\dot{V}O_2$ max. These observations support the evidence that muscle performance specifically reflects lactate threshold and not cardiovascular performance (Farina et al. 2007; Gladdon 2000). In contrast, other contractile characteristics as reflected by the rate of torque development, rate of relaxation and the changes in these measures, did not significantly relate to any measures during the exercise testing measures. There was a trend for

individuals with greater change in rate of torque development being able to keep going for longer during exercise testing. Rate of torque development characteristics have been shown to reflect underlying muscle fibre composition in animal studies (Gordon *et al.* 1990) and may reflect individuals' muscle myosin isoform profiles. Muscle contractile characteristics may give an indication of different areas of performance, with the fatigue index relating to endurance measures and the rate of torque development and rate of relaxation relating to stronger, more powerful muscular actions.

The lactate threshold, a measure of metabolic control during exercise and strongly related to endurance performance, had an inverse linear relationship with the fatigue index (Figure 4.0). Individuals with less fatigable muscles produce less lactate during exercise, have a higher lactate threshold and are able to perform at higher relative exercise intensities for prolonged periods (Coyle 2005). Therefore, the fatigue index could be utilised as a useful measure of the athlete's underlying training status.

The trend in % ΔRTD relating to PPO, with subjects displaying a higher PPO in the maximal cycle ergometer test demonstrating a greater alteration in the rate of torque development, was somewhat unexpected. It was expected that subjects able to exercise to higher power outputs would display less alteration in RTD following the fatigue protocol. However, due to heterogeneous fitness levels of the subject group, this trend in the data could be a reflection on the subjects' ability to produce powerful muscular actions during the exercise test, independent of their cardiovascular fitness. A possible factor influencing this finding is muscle fibre composition. The rate of torque development increases

from type I>IIA>IIB muscle fibres (Burke *et al.* 1973). In the present study, individuals who were unable to produce high peak powers on the cycle ergometer had muscle characteristics such that the rate of torque development was maintained throughout the fatigue protocol, with subjects reaching higher peak powers having a greater reduction in RTD. In cats, the maintenance of RTD has been shown to be characteristic of slow, highly fatigue resistant muscle fibres (Gordon *et al.* 1990) and it may be a useful indicator of muscle fibre composition in humans. Due to the length of the test the peak power attained in this study had a high aerobic contribution. If RTD is particularly related to power aspects of exercise performance it would be expected that the measure would relate significantly to more pure power events of shorter duration rather than the aerobic power test employed in this study.

The weak relationship of the muscle variables with $\dot{V}O_2$ max agrees with the findings of Sinacore *et al* (1994) in that $\dot{V}O_2$ max is a global marker of endurance capacity, affected by both central and peripheral factors. Although there was a significant relationship between the FI and lactate threshold, this may have been stronger had we employed a more controlled movement such as the knee extension protocol utilised by Garland *et al* (2004). However, the aim of this current investigation was to utilise a more dynamic exercise modality.

During voluntary progressive exercise such as that utilised in the maximal cycle ergometer test, it has been reported that muscle fibres are recruited

according to the Henneman size principle (Henneman *et al.* 1965). However, a more recent study showed that motor unit recruitment strategy is more multi-factorial and is dependent on task requirements (Hodson-Tole and Wakeling 2008). The more random recruitment patterns suggested by Hodson-Tole and Wakeling (2008) may be reflected in the weaker relationships observed between some of the contractile characteristics and exercise measures.

A possible limitation to the electrical stimulation technique utilised in the current study is the sequence of muscle fibre recruitment. Direct electrical stimulation of the muscle recruits muscle fibres in a reverse order (Trimble and Enoka 1991) or perhaps in a less organised manner (Binder-Macleod *et al.* 1995; Hodson-Tole and Wakeling 2008). Thus many factors can influence the recruitment order during electrical stimulation such as axonal branch size and arrangement within the stimulated area (Knaflitz *et al.* 1990). In light of the findings from Hodson-Tole *et al* (2008a), the recruitment pattern during electrical stimulation could be more closely related to voluntary recruitment order than first thought. Studies of voluntary fatigue have demonstrated a similar profile of torque output decreases to that reported in electrical stimulation studies (Marsden *et al.* 1983). An advantage of the electrical stimulation technique is the ability to not only assess the amount of torque drop off but also the alteration in RTD and $RR_{1/2}$ as demonstrated in this current study.

Our results demonstrate that subjects displaying a greater fatigue resistance showed a trend for a greater $\dot{V}O_2$ slow component. Garland *et al* (2004)

reported a greater $\dot{V}O_2$ slow component in power-trained subjects who displayed greater fatiguability. A possible reason for the discrepancy between the findings of this study with those of Garland *et al* is the range of subjects tested and the exercise protocols utilised to collect $\dot{V}O_2$ slow component data. The fatigue indexes of the subjects tested ranged from 36-44% in this study. This was a much smaller range, and in between the range for endurance (31.5%+/- 3.7) and power-trained (50.0 +/- 3.4) tested in Garland *et al*'s study. The greater range in Garland *et al*'s work, coupled with our more homogenous group for fatiguability tested in this study, possibly highlights the fact that the fatigue protocol is sensitive to differentiation between extreme subject populations but maybe not between those of similar training status. Also, Garland *et al* utilised a localised testing protocol of knee extensions. The aim of the testing protocol in our study was to perform the experiment in a more "real life" exercise situation. Further work is warranted to investigate this relationship in a larger subject sample.

In this investigation we observed that muscle contractile characteristics related to muscle performance markers but not to central performance markers. Specifically, from the muscle testing protocol, a smaller drop in torque during the fatigue protocol was related to an ability to exercise at higher percentages of $\dot{V}O_2$ max before the increased appearance of lactate in the blood. There was a trend for a greater drop in rate of torque development relating to achieving a higher peak power at exercise test termination. The observation of the fatigue index relating to aerobic metabolism markers, and the rate of torque development possibly relating to power performance

markers, suggests that the electrical stimulation protocol utilised in this study could be profitably used to investigate muscle responses to exercise and their contribution to overall performance. The observed test-retest stability of muscle contractile characteristics (Chapter 2) further support the utilisation of this measure within longitudinal studies and as a useful technique alongside established measures when constructing a physiological profile.

4.5 Conclusion

The fatigue index related to the LT but not $\dot{V}O_2$ max, with subjects displaying a lower fatigue index having a higher LT. This is possibly due to the peripheral components of the measures. The %ΔRTD did demonstrate a trend to relate to most determinants of endurance performance particularly peak power. This again supports the finding that muscle characteristics, collected using electrical stimulation, relate to determinants of endurance performance. Electrically stimulated markers of muscle contractile characteristics do reflect those of previously established measures. The specific relation of some of the contractile properties to endurance performance indicates the importance of muscle contractile characteristics and possible usefulness for monitoring and evaluating training. In Chapter Five the relationship of muscle performance to high intensity exercise will be investigated.

5 Muscle contractile characteristics: relationship with high intensity exercise

5.0 Summary

This Chapter will build on the findings from Chapter 4, where relationships between the muscle fatigue index and lactate threshold was found, and further assess the relationship of muscle characteristics to measures from a supramaximal exercise bout requiring more powerful muscular actions.

A significant relationship between rate of torque development and the rate and amount of fatigue during the WAnT was found. There was also a significant relationship between the muscle fatigue index and the power reduction recorded during the WAnT.

5.1 Introduction

In Chapter Four muscle contractile characteristics were found to relate to known markers of endurance performance. Interestingly the rate of torque development (RTD) seemed to relate to more power-related variables such as aerobic power, however this did not reach significance. One possible reason for this is the length of such a test required for the assessment of endurance where fatigue resistance is a greater requirement. It is possible that measures such as RTD and rate of relaxation (RR$_\frac{1}{2}$) will be more closely related to activities of shorter duration and of higher intensity. Such measures have been linked with fibre type composition (Colliander *et al.* 1988; Harridge *et al.* 1996; Trappe *et al.* 2006; Trappe *et al.* 1995). Type II, fast twitch fibres have the contractile characteristics that relate to explosive actions (Bar-Or *et al.* 1980; Thorstensson *et al.* 1977).

Although muscle biopsies do allow fibre type and enzyme content to be measured, a major limitation of the measure is that it is only reporting on a very small sample of the muscle and assuming it to be a reflection of the muscle as a whole. Although the technique can reflect previous recruitment patterns it cannot report muscle performance as a whole. Building on the findings from biopsy studies, it can be hypothesised that subjects demonstrating contractile characteristics of Type II muscle fibres, such as a faster RTD and RR($\frac{1}{2}$) and greater disruption in these parameters with exercise, would relate to performance in a high intensity bout of exercise.

The aim of this study was to investigate the relationship of muscle contractile characteristics (collected using an electrical stimulation protocol) to the main parameters from a Wingate anaerobic test (WAnT); peak power, mean power, fatigue rate and fatigue index in order to confirm the relationship of muscle contractile characteristics to exercise performance.

5.2 Method

Seventeen healthy recreationally-active subjects (13 male), mean age 29.2 $\pm$ 9.2, height 1.79 $\pm$ 0.09, weight 78.1 $\pm$ 10.9 participated in the study. After being fully informed of the risks associated with their participation, each subject gave written informed consent. The study was approved by the local University Ethics Committee and carried out according to the Declaration of Helsinki (Loff and Black 2000).

All subjects were familiarised with the testing procedures before the commencement of the study. Each subject reported to the laboratory on two occasions. The first was for the collection of muscle contractile characteristics and the second was to perform a WAnT. All tests were conducted at the same time of day (+/- 2 hrs) to reduce diurnal effects. Prior to testing, subjects

were asked to refrain from exhaustive exercise 48 hrs prior to the session, and to maintain their normal dietary habits and come to the laboratory in a euhydrated state.

5.2.1 Measurement of muscle performance

Muscle contractile measures were collected as described in 4.2.1. From this data the fatigue index, slope of the fatigue curve after 10, 20 and 60s, rate of torque development and rate of relaxation were calculated for subsequent correlation analysis with the WAnT parameters of peak power, mean power, fatigue index and fatigue rate. Rate of torque development and rate of relaxation were calculated at the beginning and end of the fatigue protocol and were calculated as described previously in 3.6.2.

5.2.2 WAnT protocol (Bar-Or et al. 1977)

The WAnT tests were conducted on a mechanically braked ergometer (Monark 824E). The ergometer was fitted with a racing saddle and toe straps and was fully adjustable for each subject. Prior to the commencement of the test the subjects completed a standard 5-minute warm up which included 3 short sprint efforts. Following the warm up subjects were allowed to stretch before the commencement of the test. Subjects were instructed to pedal as fast as possible and were given a 3 second countdown before a set resistance was rapidly applied. The individual braking load was calculated as 75 g/kg body wt (Bar-Or *et al.* 1977). The subjects were encouraged to maintain the highest possible cadence for the 30 seconds of the test. The same motivation was given to each subject. WAnT parameters were recorded and subsequently analysed using customised software (Cranlea Wingate software, Birmingham, UK). Peak power was recorded as the highest power output

(always within the first 5 seconds), mean power was the average power maintained over the duration of the test, fatigue index was calculated as the percentage drop off in torque from peak and minimum power outputs, and fatigue rate was calculated as the rate of decline in power output (W's).
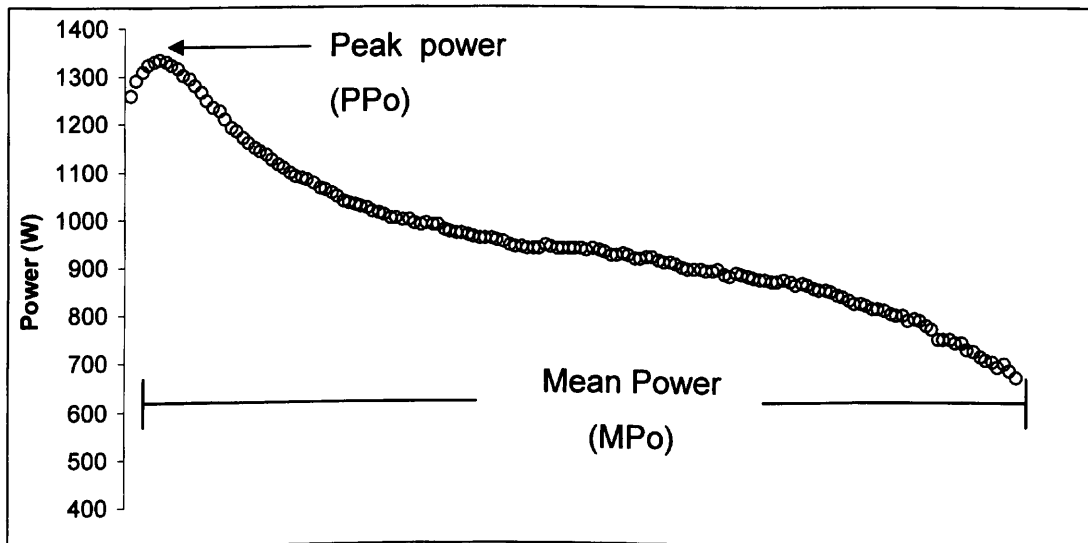
Figure 5.0 A typical power curve from a Wingate cycle ergometer test demonstrating areas of the curve used for the calculation of peak power (PPo) and mean power (MPo).

5.2.3 Statistical analysis

Descriptive statistics included mean +/- sd for all measured variables and all data was examined for normality using a Kolmogorov-Smirnoff test. Pearson's correlation coefficient analysis was used to assess relationships between the muscle characteristics and WAnT parameters. Due to multiple tests, a Bonferroni adjustment was utilised therefore statistical significance was accepted at $p < 0.01$, so as to avoid Type I error.

5.3 Results

Mean and standard deviation data for the measures taken in this study are displayed in table 5.0.

Table 5.0 Subject characteristics from the Wingate anaerobic test (WAnT) and muscle performance testing.

	Mean	SD
WAnT Peak Power (W)	732.6	149.3
WAnT Mean Power (W)	554.8	123.0
WAnT Fatigue Index (%)	41.6	12.9
WAnT Fatigue Rate (W·s)	11.1	4.5
Muscle Fatigue Index (%)	39.3	14.5
RTD (Nm·s)	447.7	158.2
RR$_{½}$ (Nm·s)	567.2	354
%ΔRTD	38.0	19.5
%ΔRR$_{½}$	61.2	18.7

WAnT Peak power = maximum power from the Wingate anaerobic test.
WAnT mean power = mean power produced during the Wingate anaerobic test.
WAnT fatigue index = max power − min power / max power x 100.
WAnT fatigue rate = -dW / dt.
Muscle Fatigue Index = max torque − min torque / max torque x 100
Rate of torque development (RTD) = dF / dt
Rate of relaxation (RR$_{½}$) = -dF peak / dt half peak
% Change in RTD = Initial RTD − Final RTD / Initial RTD x 100
% Change in RR$_{½}$ = Initial RR$_{½}$ - Final RR$_{½}$ / Initial RR$_{½}$ x 100

The muscle fatigue index related significantly to the WAnT fatigue index (Figure 5.1 p<0.01). Subjects displaying a greater fatigue index during the electrical stimulation protocol (see Chapter Three, section 3.2) displayed a higher fatigue index in the cycle ergometer test. The muscle fatigue index also related significantly to the fatigue rate (FR) during the WAnT (Figure 5.2 p<0.01).
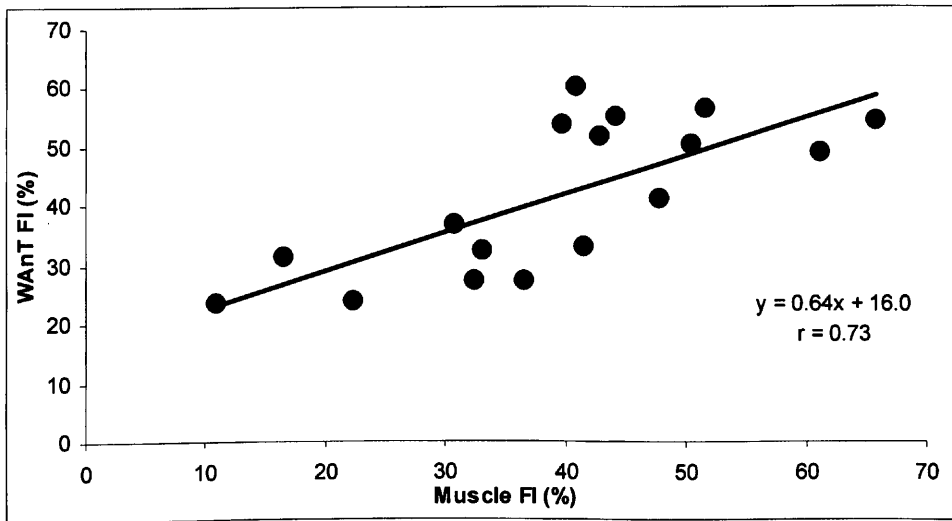
Figure 5.1 Relationship between the Wingate fatigue index (WAnT FI%) and the muscle fatigue index from the electrical stimulation protocol.
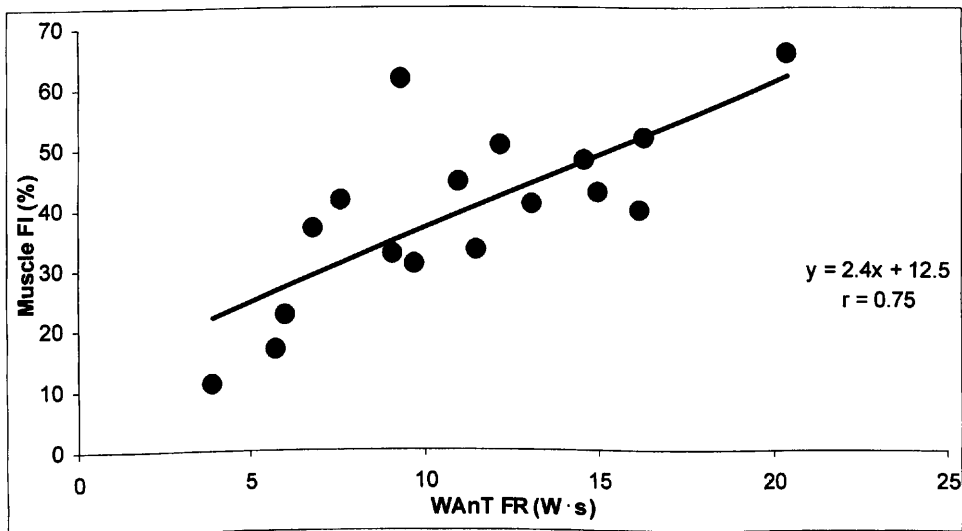
Figure 5.2 Relationship of the muscle fatigue index and the Wingate fatigue rate (WAnT FR).

The change in rate of torque development (%ΔRTD) also related significantly to the fatigue rate (W.s) during the WAnT (Figure 5.3). Subjects displaying the greatest reduction in RTD had the greatest fatigue rate during the WAnT and greater fatigue during the electrical stimulation protocol. There was no significant relationships between peak (r 0.36, p>0.01) or mean power (r - 0.11, p>0.01) with any of the muscle performance measures.
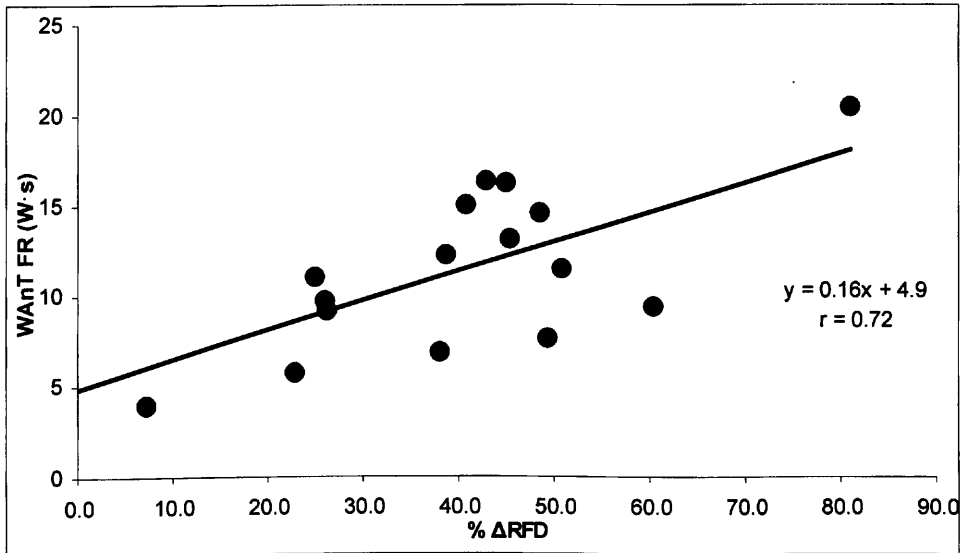
Figure 5.3 Relationship of the Wingate fatigue rate (WAnT FR) and percentage change in rate of torque development (%ΔRTD).

As with the findings in Chapter Four there were no significant relationships between the shape of the fatigue curve and the exercise measures.

5.4 Discussion

In line with the findings of Chapter Four, muscle contractile characteristics were found to relate to specific parameters of exercise performance. The finding of %ΔRTD relating to exercise performance is in contrast to the findings in Chapter Four. The stronger relationships between all muscle performance measures and the WAnT is possibly due to the greater reliance on glycolytic energy production during the protocols (Barclay and Loiselle 1992). In this current study, subjects displaying greater fatigue during the WAnT also displayed a greater drop off in torque during the muscle fatigue protocol. The relationship between the two measures (r = 0.73) was similar to that reported by Bar-Or *et al* (2003) between the WAnT fatigue index and the percentage of fast twitch muscle fibre composition (r = 0.75). Although muscle fibre type composition was not assessed directly, the contractile characteristics measured are reflective of not only the different fibre types

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within the stimulated area but also architectural parameters such as pennation angle and fibre length (Brancaccio *et al.* 2007). It is well established that fast twitch fibres are more susceptible to fatigue (Karlsson *et al.* 1981) and also changes in rate of torque development characteristics are greater in this fibre group (Gordon *et al.* 1990; Hamada *et al.* 2003). Greater decrements in knee extension exercise (Colliander *et al.* 1988) and sprint cycle ergometer performance (Bar-Or *et al.* 1980; Calbet *et al.* 2003) have been associated with greater Type II fibre type composition. These findings support the results of this current study in that the muscle contractile characteristics, collected via the electrical stimulation, are reflective of the underlying muscle fibre composition, structure and function.

It was somewhat surprising that the muscle contractile characteristics did not relate to peak and mean power measured in the WAnT. It could be hypothesised that subjects displaying higher peak power outputs in the WAnT, reflective of a greater proportion of powerful fast twitch muscle fibres (Calbet *et al.* 2003), have a greater muscle fatigue index. A possible limitation affecting the power measurements during the WAnT in this current study is the calculation of the load resistance. A common calculation of resistance is based on the original developmental work for the WAnT, that being 75 g·kg (Bar-Or *et al.* 1977). Many studies have been conducted to assess the optimised load for peak power production. The loads reported in a review by Bar-Or (1987) ranged from 80 g to 110 g·kg body wt. in attempt to maximise power generation. It was beyond the scope of this current investigation to optimise the resistive load for each individual. The load selected for this study is the current standard for electro-magnetically controlled braked cycle ergometers during successful WAnT tests (Beneke *et al.* 2002; Micklewright *et*

al. 2006). However it is possible that the load selected did not allow optimal power output for some of the subjects and therefore affected relationships to the muscle contractile characteristics.

The WAnT is a supra-maximal test and thus requires complete motivation to perform high intensity exercise. This can have a significant effect on the results. The advantage of the electrical stimulation protocol is that assessments are made independently of the subjects' motivation.

5.5 Conclusion

The results of this study demonstrate that muscle contractile characteristics relate to measures from the WAnT. The muscle fatigue and rate of torque development alterations demonstrated during the electrical stimulation protocol reflect the profile seen during the WAnT. There were significant relationships between the fatigue measures from both protocols and rate of torque development characteristics. These findings build on those in Chapter 3 and 4. Chapter 3 demonstrated the reliability of the measures and Chapters 4 and 5 have shown that the measures relate to exercise performance of varying intensity. These are the first studies that have investigated such relationships and highlight the muscle performance protocol as a useful additional tool to increase our understanding of the muscle response to exercise. Chapter 6 will utilise the muscle performance protocol to investigate the effect of exercise intensity on muscle performance measures acutely after exercise.

6 Alterations in peripheral muscle characteristics following high and low intensity bouts of exercise

6.0 Summary

The aim of this Chapter is to monitor the effects of two exercise interventions of the same total work but of different intensity on peripheral muscle. Muscle contractile characteristics were measured before and after a high and low intensity exercise interventions that were controlled for the same amount of work. There was greater disruption in muscle performance following the intermittent protocol. Following the lower intensity exercise, muscle contractile characteristics had recovered after 1 hour.

6.1 Introduction

Currently a battery of central and peripheral markers are used to individually guide endurance training (Dennis *et al.* 2008). Whilst the relation of muscle contractile characteristics, measured using transcutaneous electrical stimulation, to aerobic and anaerobic measures of exercise performance have been established (Chapters 4 and 5), to date no work has been conducted to investigate muscle contractile characteristic response to high and low intensity exercise. Muscle contractile characteristics have demonstrated to be stable and reproducible (Chapter 3) and offer an opportunity to monitor muscle recovery following exercise. The aim of this study was to monitor muscle contractile characteristics *in vivo* immediately following two distinct exercise sessions to assess changes in muscle performance between an acute high and low intensity exercise session conducted at the same average intensity.

The recovery of muscle performance up to 1 hour post-exercise was further observed.

Pilot work

Prior to the commencement of the major study, a pilot study was undertaken to assess the sensitivity of the muscle performance measures as to date no studies have reported the direct effect of exercise intensity on these changes. One male subject (age 28yrs, weight 78.8kg, height 1.88m) completed the testing procedures following giving written informed consent. He reported to the laboratory on 3 occasions. On the first visit baseline electrical stimulation data was collected for rate of torque development (RTD), rate of relaxation ($RR_{1/2}$) and the fatigue index (FI) on the right quadriceps femoris as described in section 3.2. This was followed by a maximal incremental test to exhaustion as described in section 4.2.2. In the second visit the subject completed a 30s Wingate anaerobic sprint test (WAnT) on a cycle ergometer as previously described in 5.2.2. Immediately post-WAnT the subject was seated back on the strength testing chair and the electrical stimulation protocol conducted in visit 1 was repeated. During visit 3 the subject cycled for 30 mins at an intensity equivalent to approximately $70\% \dot{V}O_2$ max calculated from the maximal test. As in the WAnT trial, the electrical stimulation protocol was performed immediately post-$70\% \dot{V}O_2$ max trial.

Pilot Results

The slope of the fatigue curve of the muscle performance test was significantly less steep following the Wingate trial at 10, 20 and 60s time points ($p<0.05$). Figure 6.0 displays the first 60s of the torque output following the different exercise interventions.
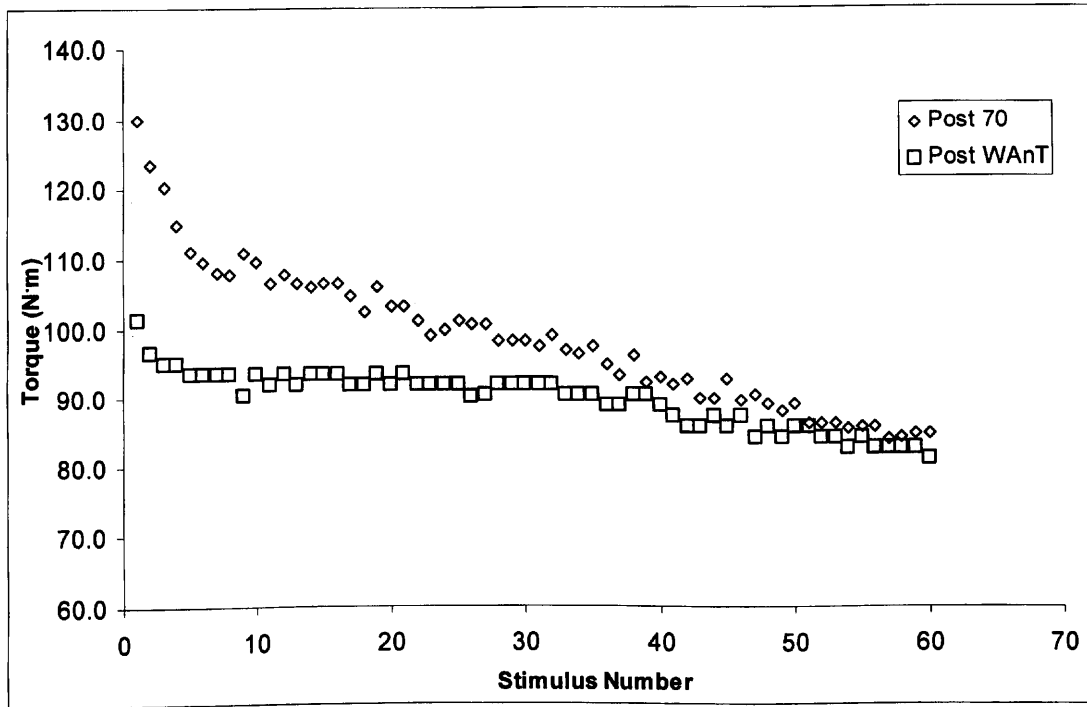
Figure 6.0 Analysis of first 60 data points of fatigue test immediately following the Wingate trial (square symbol – Post WAnT) and exercise bout at 70% $\dot{V}O_2$ max (diamond symbol – Post 70).

No statistical methods were used to assess rate of torque development (RTD) or rate of relaxation (RR½). However, there was a trend for RTD to be lower, in comparison to baseline, following the Wingate trial only. Following the exercise bout at 70% $\dot{V}O_2$ max RTD was slightly increased (Figure 6.1).

Rate of relaxation (RR½) was maintained following the Wingate trial but there was a trend for an increase following the 70% $\dot{V}O_2$ max trial (Figure 6.1).
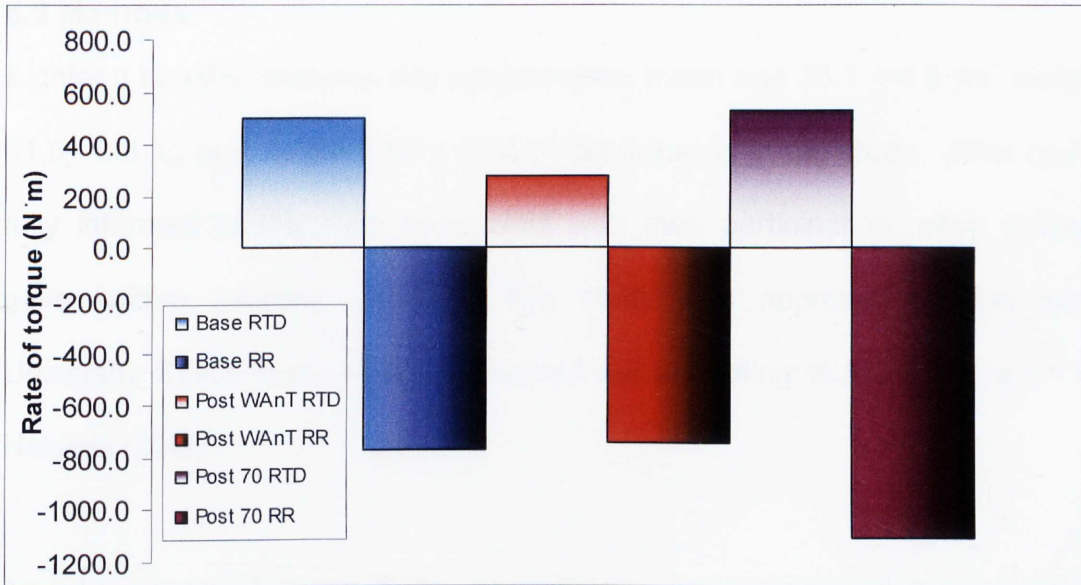
Figure 6.1 Rate of torque development and rate of relaxation in rested muscle (Base RTD/RR) and immediately following a Wingate test (Post WAnT RTD/RR) and exercise bout conducted at 70% $\dot{V}O_2$ max (Post 70 RTD/RR).

Pilot study findings

The results from this pilot study are limited in terms of the conclusions that can be made. However the findings have highlighted the possibility of utilising the measures to report the muscle response to different intensity of exercise. The findings support the need to investigate further the effects of different intensity, acute exercise interventions on the contractile characteristics in a larger sample size. Also it is not clear whether the changes in muscle contractile characteristics reported in this study were due to the intensity of the work bout or the total amount of work completed. The aim of the following work is to answer the questions raised from this pilot work, particularly controlling for total external work. The results of the larger study will help to highlight the individual peripheral muscle response to an acute training stimulus.

6.2 Methods

Eighteen healthy recreationally active males, mean age 25.1 ± 4.5 yrs, weight 81.6± 9.8 kg and height 1.83 ± 0.06 m participated in the study. After being fully informed of the risks associated with their participation, each subject gave written informed consent. The study was approved by the local University Ethics Committee and carried out according to the Declaration of Helsinki (2000).

All subjects were fully habituated to the testing procedures before participating in the study. The habituation session allowed the participants to experience the sensation of electrical stimulation. Subjects unfamiliar with the methods for collecting expired air were familiarised with the technique during this session. Following habituation, subjects attended three assessments one week apart (Figure 6.2). In assessment 1 baseline muscle performance and $\dot{V}O_2$ max data was collected. Assessments 2 and 3 consisted of either a high or low intensity bout of exercise. The order of testing sessions 2 and 3 were randomised. Muscle performance data was collected immediately after each of the exercise interventions in assessments 2 and 3 and after 1 hour post-exercise in 10 subjects. To minimise any diurnal effects all sessions were conducted at the same time of day (+/- 2 hrs). Prior to testing, subjects were asked to refrain from exhaustive exercise 48 hrs prior to the session, and to maintain their normal dietary habits arriving at the laboratory in a euhydrated state.
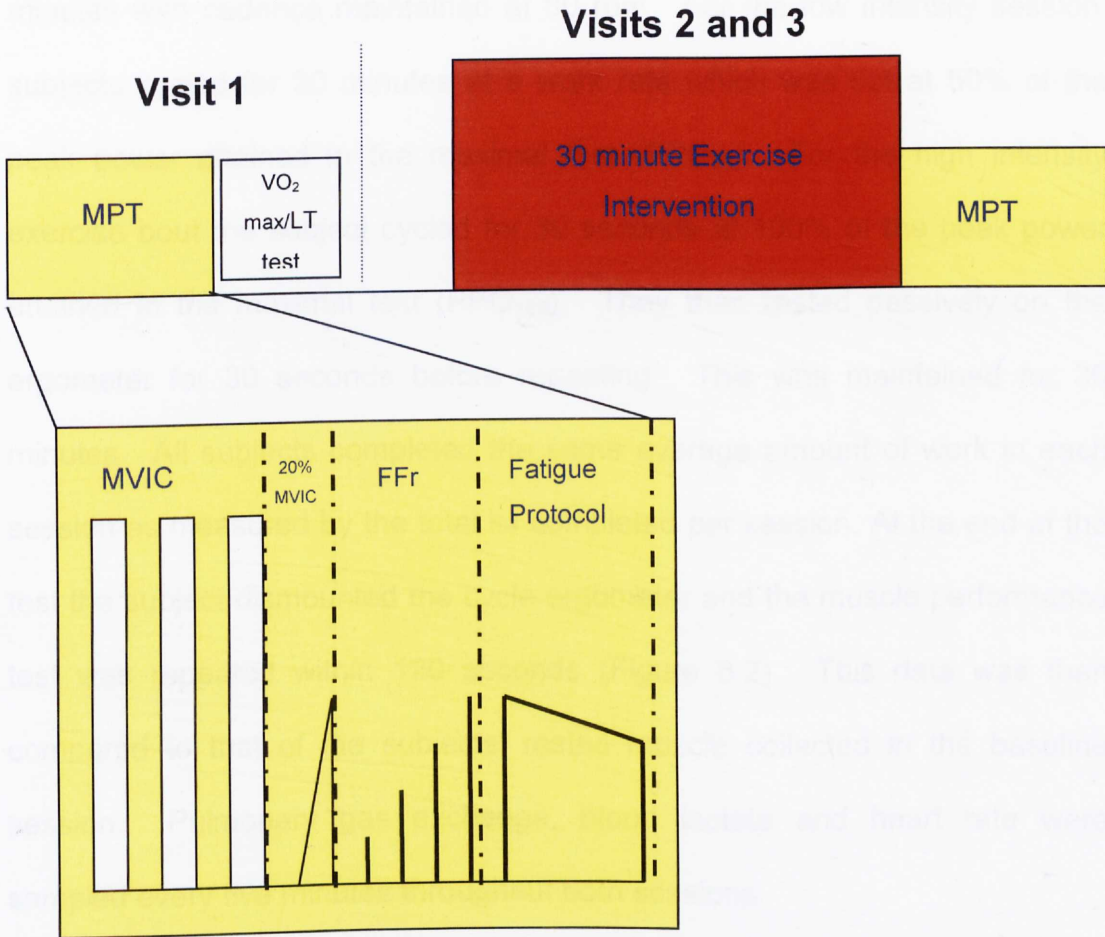
Figure 6.2 Schematic of testing sessions.

MPT = muscle performance test; MVIC = maximal voluntary isometric contraction.

20% MVIC = electrical stimuli required to elicit a force equal to 20% of MVIC.

FFr = torque outputs at stimulus frequencies at 1, 10, 20 and 40Hz.

Fatigue protocol = 3 minute stimulation protocol at 40Hz.

VO$_2$ max/LT test = Maximal oxygen uptake and lactate threshold collected from the incremental cycle test.

6.2.1 Measurement of muscle performance and exercise parameters

The methods for collection of muscle contractile characteristics, peak oxygen consumption ($\dot{V}O_2$ max) and lactate threshold (LT) are described in chapter 4 (4.2.1 and 4.2.3 respectively).

6.2.2 Exercise interventions

On two occasions, separated by 1 week, each subject completed a high and low intensity exercise session. The order in which the subjects performed the sessions was randomised. Both sessions involved cycling on a cycle ergometer (Lode Excalibur Sport. Lode, Gronigen, The Netherlands) for 30

minutes with cadence maintained at 80 rpm. For the low intensity session, subjects cycled for 30 minutes at a work rate which was set at 50% of the peak power attained in the maximal test (PPO_{50}). For the high intensity exercise bout the subject cycled for 30 seconds at 100% of the peak power attained in the maximal test (PPO_{100}). They then rested passively on the ergometer for 30 seconds before repeating. This was maintained for 30 minutes. All subjects completed the same average amount of work in each session as measured by the total kJ completed per session. At the end of the test the subject dismounted the cycle ergometer and the muscle performance test was repeated within 120 seconds (Figure 6.2). This data was then compared to that of the subjects' rested muscle collected in the baseline session. Pulmonary gas exchange, blood lactate and heart rate were sampled every five minutes throughout both sessions.

6.2.3 Reliability of change data

Ten subjects returned to the laboratory to repeat the high and low intensity exercise sessions to assess the reliability of the post exercise muscle performance measures. During these trials muscle performance data was collected immediately post exercise as previously described. Muscle temperature was also indirectly assessed using skin temperature probes placed over the region of the vastus lateralis during both exercise trials.

6.2.4 Data analysis

Calculation of fatigue resistance, rate of torque development (RTD), rate of relaxation ($RR_{1/2}$), torque frequencies and the assessment of high and low frequency fatigue for pre- and post- exercise interventions were analysed as previously described in chapter 3 (3.6). From curve estimation a linear fit was

found to be the best fit to the data, determined on the strength of the correlation coefficient, and used for the analysis of the slope of the fatigue curve at 10, 20 and 60 s following both exercise trials.

6.2.5 Statistical analysis

Descriptive statistics of mean +/- sd for all variables were determined. Repeated measure analysis of variance with within subject factors for exercise task (3 levels) was used to assess any differences between the baseline muscle data and that following the high and low intensity exercise interventions. Where significance was indicated, Bonferonni post-hoc analysis was utilised to examine which of the means were significantly different. Paired student t tests were used to assess any differences in the repeated post exercise muscle performance data between the exercise trials. Dummy variable intervention analysis was utilised to assess any differences in the slope and intercepts of the fatigue curves. The level of significance was set at $p < 0.05$.

6.3 Results

Subject exercise characteristics are displayed in Table 6.0. There was no significant difference between the amounts of work completed during the two exercise sessions (p>0.05).

Table 6.0 Subject characteristics (mean and (sd)) for maximal voluntary isometric contraction (MVIC) at rest, maximal oxygen uptake ($\dot{V}O_2$ max) and work completed during the high (Work$_{100\%}$) and low (Work$_{50\%}$) intensity exercise bouts.

MVIC (N·m)	$\dot{V}O_2$ max (L·min^{-1})	Work$_{50\%}$ (kJ)	Work$_{100\%}$ (kJ)
246.1	4.22	305.4	306.4
(74.2)	(0.58)	(39)	(39)

MVIC = Maximal voluntary isometric contraction.

VO$_2$ max = Maximal oxygen uptake, measured in the incremental cycle ergometer test.

Work$_{50\%}$ = Work intensity for the continuous exercise trial, set at 50% of the peak power reached in the incremental cycle ergometer test.

Work$_{100\%}$ = Work intensity for the intermittent exercise trial, set at 100% of the peak power reached in the incremental cycle ergometer test.

6.3.1 Maximal voluntary isometric contraction (MVIC)

Both exercise interventions led to a significant reduction in MVIC in comparison to baseline, post-low (p 0.02) and post-high (p 0.001) (Table 6.1). The post-high MVIC was significantly lower than post-low (p 0.01).

Table 6.1 Muscle performance measures at rest (Base) and following the high (post 100%) and low (post 50%) intensity exercise trials.

	Base	Post 100%	Post 50%
MVIC (N·m)	246.1 (74.2)	207.1 (67.6)*#	227.5 (65.0)*
Fatigue Index (%)	39.1 (6.5)	33.9 (12.3)*	31.9 (6.8)*
RTD (N·m·s)	232.3 (62.3)	129.4 (46.1)*#	178.6 (61.4)*
RR$_{1/2}$ (N·m·s)	-322.4 (115.0)	-189.6 (95.8)*#	-252.1 (153.3)*
10:40 ratio (%)	54.6 (13.1)	34.7 (16.0)*	41.3 (14.6)*
20:40 ratio (%)	85.9 (5.4)	71.5 (13.3)*#	79.4 (10.1)*

MVIC = Maximal voluntary isometric contraction.
Fatigue Index = max torque – min torque / max torque x 100.
Rate of torque development (RTD) = dF / dt.
Rate of relaxation (RR$_{1/2}$) = -dF peak / dt half peak.
10:40 ratio = Ratio of torque output produced at 10Hz stimulation to torque output produced at 40Hz.
20:40 ratio = Ratio of torque output produced at 20Hz stimulation to torque output produced at 40Hz.
(* significant from baseline, # significant from low) $p<0.05$

6.3.2 Rate of torque development (RTD)

RTD immediately following the exercise sessions was significantly reduced following both interventions. The reduction was greatest following the high intensity intervention (p 0.001) (Table 6.1).

6.3.3 Rate of relaxation (RR $_{1/2}$)

RR$_{1/2}$ immediately following the exercise sessions was significantly slower following both exercise interventions in comparison to baseline (Table 6.1). The greatest reduction was after the high intensity intervention (p 0.001).

6.3.4 Torque frequencies

There was a significant drop in all of the torque frequencies following both interventions (Figure 6.3). The reduction in the torque frequencies was significantly greater following the high intensity exercise.
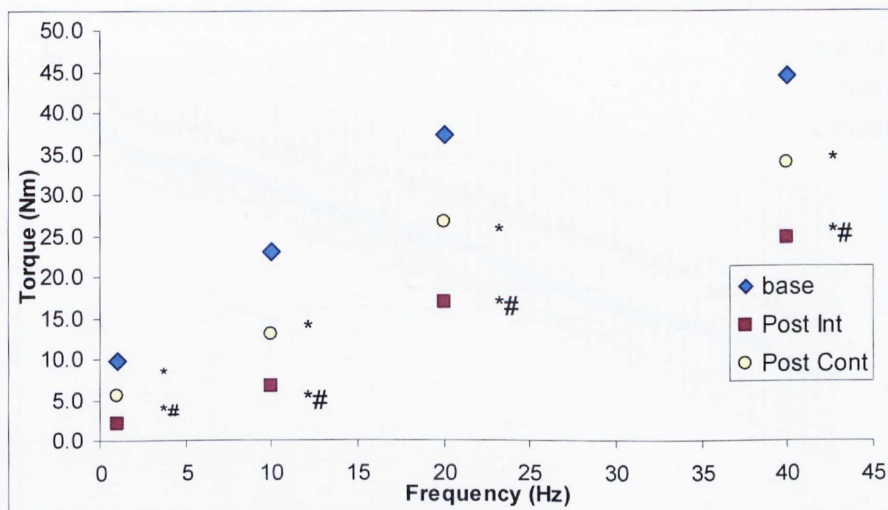
Figure 6.3 Changes in absolute TFR. (· Significantly different to baseline # Significantly different from continuous exercise bout)

6.3.5 High and low frequency ratios

Both low (p 0.001) and high (p 0.001) intensity exercise caused a significant reduction in 10:40 Hz and 20:40 Hz ratios. There was no difference between the interventions for 10:40 but there was a significant difference at 20:40 (p 0.005).

6.3.6 Central measures

There was also a significant drop in the ratio between maximal voluntary torque and electrically stimulated torque at 40 Hz. High intensity exercise caused a greater reduction in this ratio (p 0.001).

6.3.7 Fatigue curve slope analysis

Both exercise interventions resulted in a significant reduction in the slope of the fatigue curve in comparison to resting baseline measures (p<0.01). There was also a significant reduction in the slope following the high intensity intervention compared with the low intensity exercise (p<0.01). Figure 6.4 illustrates the 60 s slope at baseline and post exercise interventions.

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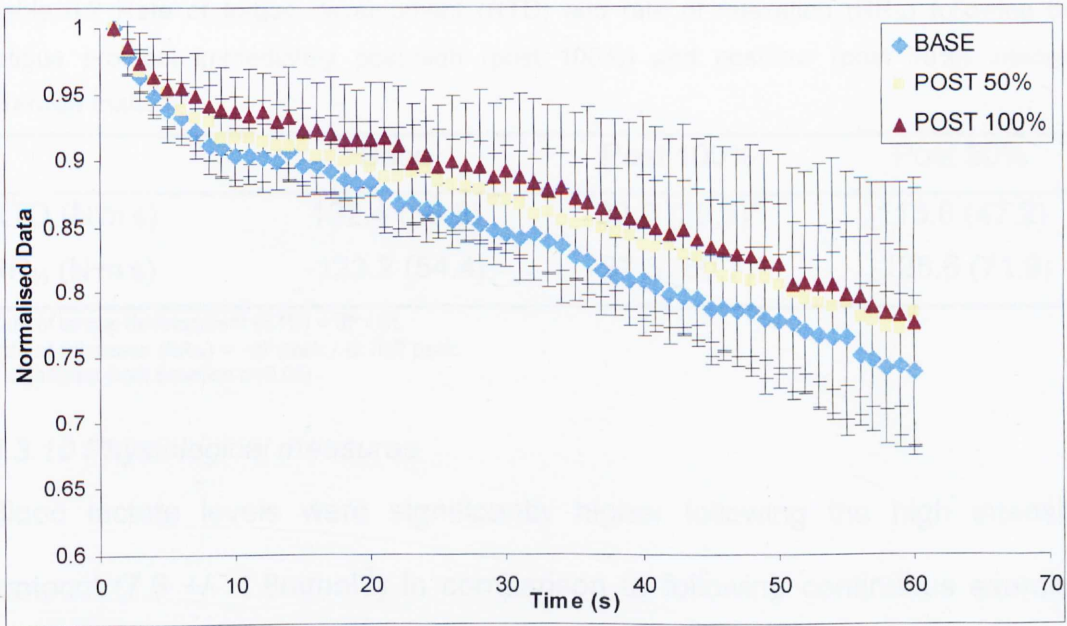
Figure 6.4 First 60s slopes from the fatigue protocol at baseline (Base), post-low (Post 50%) and post-high (Post 100%) intensity exercise interventions.

6.3.8 Fatigue resistance and RTD and RR following fatigue test

The fatigue index was significantly reduced following exercise (p 0.05) in comparison to baseline. There was no significant difference between the two interventions.

6.3.9 Changes in rate of torque development (RTD) and rate of relaxation (RR½) following the fatigue test.

Following the high intensity exercise session both the rate of torque development and relaxation were further significantly reduced following the fatigue protocol compared to baseline with no change following the continuous session (Table 6.2).

Table 6.2 Rate of torque development (RTD) and rate of relaxation (RR$_{\frac{1}{2}}$) following the fatigue protocol immediately post-high (post 100%) and post-low (post 50%) intensity exercise trials.

	Base	Post 100%	Post 50%
RTD (N·m·s)	132.4 (38.0)	82.0 (39.5)*	113.8 (47.2)
RR$_{\frac{1}{2}}$ (N·m·s)	-123.2 (54.4)	-97.8 (80.7)*	-125.6 (71.9)

Rate of torque development (RTD) = dF / dt.
Rate of relaxation (RR$_{\frac{1}{2}}$) = -dF peak / dt half peak.
(* significant from baseline p<0.05)

6.3.10 Physiological measures

Blood lactate levels were significantly higher following the high intensity protocol (7.6 +/- 1.8mmol^{-1}) in comparison to following continuous exercise (2.6 +/- 1.5mmol^{-1}) (p< 0.001). Oxygen uptake was significantly different between the protocols, 2.91 (+/- 0.45) L·min^{-1} and 2.67 (+/- 0.29) L·min^{-1} for the high and low intensity sessions respectively (p< 0.02). Heart rate averaged 82.9 (3.1) % and 74 (4.4) % of maximum heart rate during the high and low intensity protocols respectively.

6.3.11 Reliability of post-exercise muscle performance data.

In the ten subjects that repeated the exercise trials there were no significant differences in any of the muscle performance measures following either high or low intensity exercise interventions, with interclass correlation coefficient values ranging from 0.78 to 0.98 following the low and high intensity sessions respectively. Average skin temperature was 27.9$^{\circ c}$ +/- 0.9 and 28.2$^{\circ c}$ +/- 0.7 (p>0.05) following the high and low intensity protocols respectively.

6.3.12 Recovery of muscle responses at 1 hour post-exercise.

Absolute torque frequency responses were not significantly different to baseline levels 1 hr post-low intensity exercise. However, torque frequencies remained significantly lower 1 hr post-high intensity exercise. Table 6.3

displays the recovery of the absolute torque frequency profile immediately post exercise and 1 hr post exercise.

RTD was significantly reduced immediately following both exercise interventions, with a greater reduction following high intensity exercise. However, RTD was not significantly different to baseline levels 1 hr post-low intensity exercise but remained lower following the high intensity protocol.

Table 6.3 Recovery of rate of torque development (RTD) and rate of relaxation (RR$_{\frac{1}{2}}$) immediately post-high (post 100%) and post-low (post 50%) and at 1 hour post (post-1hr 100% and post 1-hr 50%) (Mean (SD)).

	Base	Post 100%	Post-1hr 100%	Post 50%	Post-1hr 50%
RTD (N·m·s)	288	127.0*#	169.4*#	226.7*	239.9
	(71.5)	(89.8)	(98.6)	(120)	(88.6)
RR$_{\frac{1}{2}}$ (N·m·s)	-472.2	-221.5*#	-251.2*	-401.4	-371.5*
	(114.9)	(170.1)	(182.0)	(210.2)	(171.1)
1 Hz TFR	34.9	5.7*	13.2*#	20.1*	28.8
N·m	(6.8)	(3.5)	(5.3)	(8.2)	(10.4)
10 Hz TFR	72.6	13.1*#	25.3*#	35.4*	45.9
N·m	(22.5)	(9.6)	(10.9)	(23.0)	(27.4)
20 Hz TFR	108.1	30.8*#	47.2*#	68.7*	84.7
N·m	(20.9)	(17.2)	(19.3)	(29.8)	(38.8)
40 Hz TFR	126.6	43.2*#	62.3*#	91.0*	103.8
N·m	(23.5)	(20.2)	(24.5)	(35.8)	(42.1)

Rate of torque development (RTD) = dF / dt.
Rate of relaxation (RR$_{\frac{1}{2}}$) = -dF peak / dt half peak.
1 Hz TFR = Torque frequency at 1 Hz.
10 Hz TFR = Torque frequency at 10 Hz.
20 Hz TFR = Torque frequency at 20 Hz.
40 Hz TFR = Torque frequency at 40 Hz.
(* significant from baseline; # significant from low $p < 0.05$)

Following low intensity exercise $RR_{1/2}$ demonstrated a delayed response. Immediately post-low intensity exercise there was no significant difference with baseline however, at 1 hr post-low there was a significant reduction in relaxation time. Following the high intensity protocol there was a significant reduction in $RR_{1/2}$ immediately post-exercise and this remained at 1 hr post. At 1 hr post there was no significant difference in relaxation times between the exercise interventions (Table 6.3).

6.4 Discussion

The findings of this study indicate that muscle performance was reduced following both the high and lower intensity exercise protocols but with a greater reduction in MVIC, RTD, $RR_{1/2}$, the 60 s slope of the fatigue protocol and torque frequency response following the high intensity bout, although there was no difference in fatigue resistance. Muscle performance remained reduced 1 hour following high intensity exercise but was recovered following low intensity exercise. Muscle performance may thus be a useful marker of recovery following training sessions.

Following the exercise interventions MVIC was significantly reduced. In contrast to our current findings, Lepers *et al* (2007) reported similar MVIC reductions following both constant and variable intensity cycle exercise. Although this differs from the findings in this current study, the lack of change reported in the Lepers study was probably due to smaller range of work loads utilised. Studies using a wider range of work loads, some until exhaustion, have demonstrated reductions in voluntary torque similar to ours (Stewart *et al.* 2008; Theurel and Lepers 2008), possibly due to a disruption in neuromuscular propagation (Millet *et al.* 2003).

The reduction in the ratio between MVIC and 40 Hz stimulation is an indication that the reduction in torque output is due to peripheral mechanisms within the muscle and not central drive (Place *et al.* 2004). Although no direct measure of central fatigue was taken, the results of the current study indicate that high intensity exercise places a greater training stimulus on the peripheral muscle and therefore could lead to greater adaptation. This finding is supported by the significant drop in the ratio between maximal voluntary torque and the torque output at 40 Hz. The peripheral cause of the reduction in torque could be due to a number of mechanisms including a reduction in Na^+-K^+ATPase activity (Aughey *et al.* 2007) and impaired SR Ca^{2+} release and uptake (Leppik *et al.* 2004). Although the intensity employed by Leppik *et al.* (2004) was classed as submaximal, it was prolonged and until exhaustion making comparisons with the acute interventions utilised in our current study difficult.

The fatigue index utilised in this current demonstrated similar reductions following both exercise bouts. The lack of sensitivity for this measure to report alterations in muscle performance is in accordance with the findings of (Sinacore *et al.* 1994). However, analysis of the first 60 s of the fatigue curve did highlight differences between the exercise bouts. Our findings demonstrate that the fatigue curve displayed a less steep reduction in torque following the high intensity exercise bout (Figure 6.4). This supports the findings reported in the pilot work following the high intensity bout, where the fatigue curve demonstrated a significantly reduced slope. If the first section of the fatigue curve is reflective of Type II fibre recruitment (Snyder-Mackler *et al.* 1993), it would be expected that the torque output curve from the fatigue

test following exercise would demonstrate a shallower curve following intermittent exercise due to the greater fatigue in the Type II muscle fibre pool from the preceding bout. Snyder-Mackler *et al* (1993) proposed that the reduced torque drop off seen in their patients was due to a selective atrophy of Type IIB fibres. It is possible that the higher intensity exercise bout fatigued Type IIB fibres in this study.

Following low intensity exercise, RTD and $RR_{1/2}$ were maintained following the fatigue stimulation protocol. However, high intensity exercise led to a decrease in RTD and $RR_{1/2}$. Matsunaga *et al* (2007) reported an increase in sarcoplasmic reticulum (SR) Ca^+ sequestering capacity following high intensity training, and the high intensity bout was conducted at similar intensities to that described in this study. Whilst not investigated in this study, it is probable that the higher intensity bout caused greater disruption to this mechanism. Alterations in SR functioning has been shown to relate to force development (Tupling *et al.* 2000) and this was possibly demonstrated at the end of the fatigue test following high intensity exercise, with RTD and $RR_{1/2}$ continuing to decrease and remain significantly lower than baseline and that following low intensity exercise. The capacity of the muscle to maintain its contractile characteristics after the fatigue protocol following low but not high intensity exercise can also have important performance and training implications, particularly when monitoring athletes in multi-discipline sports, as it offers the ability to measure the recovery of the muscle contractile characteristics of the individual.

Although torque frequencies were reduced following both high and low intensity interventions, torque frequencies were significantly lower following

the high intensity session. This is in agreement with the findings of Skof *et al* (2006a) and Ratkevicius *et al.* (1995) who both demonstrated reductions in high and low frequency force output following intermittent exercise. However, the findings from this study differ to those of Skof *et al* (2006b) in finding a reduction in muscle torque frequency data after both continuous and intermittent exercise. This could have been due to the lower fitness levels of the participants involved in this study, and the exercise modality. Running, as used by Skof *et* al (2006a, b) imposes different forces on the musculature due to stretch-shortening actions and has been shown to induce greater low frequency fatigue (LFF) (Skof *et al*, 2006b). Depression of the lower torque frequencies, termed low frequency fatigue (LFF), has been associated with disruption in the excitation-contraction coupling mechanism (Edwards *et al.* 1977a). When considering the ratio of torque outputs of low to high frequencies at 10:40 and 20:40 ratios there was evidence of LFF following exercise which was most pronounced following the high intensity protocol. High frequency fatigue is associated with a drop in torque outputs at higher frequencies possibly suggested to be due to a disruption in muscle membrane excitability (Edwards *et al.* 1977a; Fowles *et al.* 2002). Although we had no direct measure of membrane excitability in this study, the greater reduction in MVIC following high intensity exercise, as well as the reduction in torque frequency response, suggests that high intensity exercise caused a greater reduction in both central and peripheral drive to the muscle.

The significant level of lactate accumulation reported following the high intensity bout in this current work is evidence of the higher glycolytic demand placed on the muscle during high intensity work. Disturbance to metabolic

homeostasis within the muscle disrupts the functioning of energy consuming mechanisms such as the Na^+-K^+ATPase and Ca^+ATPase (Allen et al. 2008; Fowles et al. 2002). Metabolic products, such as lactate and inorganic phosphate, have a detrimental effect on the functioning of muscle contractile properties such as rate of torque development and rate of relaxation (Fowles et al. 2002; Leppik et al. 2004; Matsunaga et al. 2007) and is possibly reflected in the greater disruption in RTD following the high intensity protocol. However, the lactate concentrations reported following the exercise interventions demonstrated no relation to any muscle contractile characteristics following either exercise trial; therefore other mechanisms could be contributing to the reduction in muscle performance.

The intensity of exercise played a key role in the recovery profile of the muscle performance, despite the control of total work. Studies monitoring the recovery of muscle performance following exercise have reported a range of recovery periods from 120 minutes (Skof and Strojnik 2006b) up to 72 hours post exercise (Skurvydas et al. 2007). The differing recovery periods reported in these studies are a reflection of the effects of differing intensities and work loads on muscle recovery. To date this current work is the first to control for total work which focused on the effects of exercise intensity. As demonstrated in the results, following high intensity exercise a period greater than 1 hour is required for full muscle performance recovery. Whilst the same amount of work was completed in each session, the different recovery periods indicates that the measurement of muscle performance may be a useful tool to individually direct training session recovery periods.

In sports such as triathlon it is crucial that the muscles of the lower extremities can still function optimally following the cycling section of the sport. It has already been demonstrated that constant versus variable cycling affects fibre recruitment patterns (Palmer *et al.* 1999). The use of the muscle performance test following various acute exercise bouts could help our understanding of muscle contractile characteristics in subsequent exercise bouts and whether the subject is recovered.

No difference was observed in skin temperature between either of the exercise interventions in this study. This finding suggests that changes in skin temperature, used as an indirect measure of muscle temperature, would have had the same effect on muscle contractile characteristics during both trials. Ranatunga *et al.* (1987) examined the relationship between skin and muscle temperature, demonstrating a good relationship between skin and muscle below intramuscular temperatures of $20^{\circ c}$. Although their work demonstrated discrepancies between and skin and muscle temperature measurements above $20^{\circ c}$ it is presumed that this discrepancy will have been consistent in both of the exercise trials conducted in this study.

6.5 Conclusion

The measures of muscle performance, particularly rate of torque development, rate of relaxation and the slope of the fatigue curve, are sensitive to exercise intensity and could be a useful tool to gain further insight into *in vivo* muscle characteristics following exercise interventions. Muscle function was significantly reduced following high intensity exercise in comparison to lower intensity exercise even when controlled for average work completed. In a small cohort the torque frequency response had returned to

baseline levels following the low intensity protocol after one hour. Muscle performance remained reduced following the high intensity protocol. This study is the first to demonstrate the ability of such techniques to assess the muscle response to different exercise intensities. The different recovery profiles following the sessions highlight its usefulness in monitoring recovery from exercise sessions. The findings from the study also offer the further refinement to the electrical stimulation protocol developed throughout this thesis. Although the calculated fatigue index did not differ between the exercise trials, the slope of the first 60 s of the fatigue protocol was sensitive to the intensity of the previous exercise bout. This could further lend itself as a useful tool to establish the effects of various exercise intensities, recovery periods and interventions on muscle function and adaptation.

7 General conclusion

7.0 Summary

This Chapter highlights the main findings from the thesis. Specific muscle contractile characteristics related to exercise performance, with exercise intensity playing a key role in the modulation of muscle contractile characteristics. The muscle performance test was seen to be a useful marker of muscle performance and could be a useful additional tool for assessing factors relating to exercise performance. Limitations to the work and Implications for future research are discussed.

7.1 Main findings

The thesis examined the effects and relationships of exercise intensity on muscular performance. To undertake this, a muscle performance protocol using electrical stimulation was devised with the protocol refined throughout the studies. Relationships to exercise performance markers were established and the effects of exercise intensity assessed. Muscle performance was reduced following the exercise bouts and this was intensity dependent. The use of direct muscle stimulation to measure contractile characteristics can thus provide important information on the effects of exercise intensity on the exercising musculature. Chapter Three demonstrated the reliability of the stimulation protocol and the analysis procedures to be utilised throughout the thesis. In Chapter Six the measures of muscle performance were also demonstrated to be reliable when monitoring changes in muscle performance following exercise. To my knowledge this the first study to report this. The measurements could be a useful addition to physiological tests for the assessment of muscle performance and its role in athletic performance.

7.2 Discussion

Chapters Four and Five are, to the author's knowledge, the first studies to relate *in vivo* muscle contractile characteristics to functional exercise performance. Garland *et al* (2004) reported a significant relationship between the $\dot{V}O_2$ slow component and the muscle fatigue index. The work in Chapters Four and Five of this thesis build on these findings to report the relationship between the muscle contractile characteristics and exercise performance, the lactate threshold and peak power (Chapter Four) and the Wingate fatigue index (Chapter Five), during cycling ergometry. These findings highlighted the utility of this measured muscle response and the possibility of utilising these measures as subject descriptors within a battery of physiological tests, possibly for the assessment of athletic potential.

In Chapter Two the relevant anatomy and function of the muscle group investigated in this thesis (the quadriceps femoris) was discussed, and the importance of muscle architecture on muscle function highlighted. A major point raised was the variability of muscle architecture, such as pennation angle varying depending on the distance from the aponeurosis, and during some movements muscle fibres acting as force generators or transferors (Blazevich *et al.* 2006). Muscle architecture can thus have significant implications for the conclusions made on muscle assessment. Considering the issue in this thesis, muscle pennation angle was not measured, but factors such as muscle length and contractile speed were carefully controlled by the position of the subject on the strength testing chair and the use of controlled stimuli. The technique developed and utilised throughout this thesis may be

useful when describing changes in muscle contractile characteristics when attempting to assess muscle function, as a whole, following exercise.

A pilot study, presented in Chapter Six, highlighted the possibility of exercise of different intensity altering muscle contractile characteristics developed in Chapter Three. Following a Wingate Anaerobic test (WAnT), muscle contractile characteristics were substantially affected particularly for rate of torque development (RTD). Measures of RTD were greatly reduced in comparison to both baseline and following a second bout of exercise conducted at 70% $\dot{V}O_2$ max. Interestingly, the rate of relaxation of the muscle seemed unaffected by the WAnT protocol but increased following the 70% $\dot{V}O_2$ max protocol. The study reported the possibility that exercise intensity affected different components of the muscle contractile mechanism. Previous studies have shown changes in muscle performance using stimulation techniques (Lepers *et al.* 2000; Millet and Lepers 2004; Place *et al.* 2004; Skof and Strojnik 2006a; 2006b). However these studies have only looked at the effects of single exercise sessions and also some of the sessions have caused significant muscle damage (Skurvydas *et al.* 2007). In recent work, Theural *et al* (2008) have shown that when exercise intensity is controlled, greater muscular fatigue is evident immediately after variable over continuous exercise bouts. The main work in Chapter Six is the first to confirm these findings using direct muscle stimulation immediately and at 1 hour post-exercise. There were significant reductions in maximal voluntary isometric contractions (MVIC), RTD, rate of relaxation (RR½) and an increase in high frequency fatigue (HFF) following both exercise interventions but these were

significantly greater following the high intensity exercise bout, despite the same average amount of work being completed in both sessions. The muscle fatigue protocol demonstrated no further change in RTD and $RR_{\frac{1}{2}}$ following the low intensity exercise intervention but there were further significant reductions following the high exercise intervention. Of interest is that muscle performance had recovered after 1 hour post low intensity but remained reduced 1 hour post high intensity exercise, thus offering the possibility of utilising the muscle performance test as an additional measure to report the response to exercise.

The muscle performance test offers a unique method for monitoring muscle contractile characteristics. Chapter three examined the reliability of the measures utilised to report muscle performance. The findings are in line with those reported in the scientific literature using electrical stimulation techniques (Gerrits *et al.* 2001; Hanchard *et al.* 1998). Hanchard *et al* (1998) used similar stimulation intensities to those used in this thesis (approximately 20-30% MVC) and reported stable measures of both high and low frequency fatigue in rested and fatigued muscle. Although this work was conducted on a smaller muscle (tibialis anterior) their findings support those reported in this thesis on the quadriceps femoris. The protocol utilised throughout this thesis is limited to being able to report contractile characteristics only and is unable to report on other factors affecting muscle performance such as metabolism (Vanderthommen *et al.* 2003), muscle architecture (Blazevich *et al.* 2006) and muscle recruitment patterns (Adams *et al.* 1993; Hamada *et al.* 2004; Knaflitz *et al.* 1990). Studies utilising various stimulation parameters have reported varying central contributions to muscle performance (Collins 2007; Feiereisen

et al. 1997; Lagerquist *et al.* 2009). Although central contribution to muscular performance is altered depending on the stimulation pattern, the protocol remained the same throughout this thesis and demonstrated to be a reliable measure of muscle performance.

7.2.1 Limitations and future work

The work in this thesis has highlighted the possibility of using the muscle performance protocol to assess the impact of exercise on muscle contractile characteristics. These findings however should be taken in light of a number of limitations related to the electrical stimulation technique utilised and where further work is required. Firstly, as raised in the discussion in Chapter four, the muscle recruitment order during electrical stimulation is as yet not completely understood. Some research groups argue that electrical stimulation leads to a reverse recruitment of muscle fibres compared to voluntary contraction (Trimble and Enoka 1991), a more synchronous recruitment pattern (Adams *et al.* 1993) or similar to that of voluntary recruitment patterns (Hodson-Tole and Wakeling 2008). Studies have indicated that voluntary recruitment patterns could be replicated using manipulations of the pulse parameters of the electrical stimulation protocol. Although beyond the scope of this thesis, further work using various pulse parameters to attempt to better imitate voluntary contraction could lead to better relationships between muscle performance and exercise performance.

The alterations in muscle performance following the high intensity exercise in Chapter six demonstrate the usefulness of the technique for furthering our understanding of the impact of exercise intensity on muscle adaptation. However, some of the findings are dependant on a number of assumptions

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which were not measured during this work. The first is the involvement of particular muscle fibre type during the muscle performance test. Work has reported the more superficial arrangement of Type II muscle fibres in muscle (Gregory and Bickel 2005). With the percutaneous application of the electrical stimulation it could therefore be assumed that there is a greater involvement from this fibre type during the muscle performance test due to their proximity in relation to the electrodes. Exercise of high intensity has demonstrated a greater involvement of Type II muscle fibres (Casey *et al.* 1996) and this is possibly reflected in the slope of the fatigue curve following the exercise in Chapter six and in the studies of others (Snyder-Mackler *et al.* 1993). However, this hypothesis has yet to be investigated with the direct comparison of the fatigue curve changes with alterations in muscle biopsy samples. The use of muscle biopsies could also improve our knowledge of what is causing the alterations in the muscle performance test, such as the rate of torque development and rate of relaxation evident in Chapter six and the possible role of Na^+ and K^+ alterations on these measures. Although muscle biopsies offer an opportunity to directly investigate the muscle fibre it does not indicate the contraction characteristics of the muscle as a group. Although the electrical stimulation protocol does monitor the collective action of the muscle group as a whole it cannot differentiate if the alterations in muscle performance are cellular or architectural in origin. The work of Brancaccio *et al* (2007) reported muscle architectural changes following a high intensity exercise bout and this measurement alongside the muscle performance protocol utilised in this thesis could help our develop our understanding of the responses seen in muscle performance following the acute exercise sessions in Chapter six.

One possible confounding variable from the work in Chapter six is the intermittent nature of the high intensity exercise bout. The on-off nature of the exercise bout could also impact on the subsequent muscle performance. To control for this, future studies should attempt to use a continuous mode of exercise for both the high and low intensity bouts with the high intensity bout performed for a shorter period to match for total work between the bouts. This would clarify if the alterations in muscle performance following the high intensity bout were purely due to the intensity and not the on-off nature of recruitment during intermittent exercise.

With the reliability of the muscle performance test established in this thesis the use of another non-invasive measure, near-infrared spectroscopy (NIRS), performed simultaneously with the muscle performance test could help improve our understanding of the metabolic alterations during the muscle performance test. This could lead to the development of a comprehensive non-invasive tool for assessing muscle performance alterations relating to both contractile and metabolic function. Preliminary work has suggested oxygenation patterns during the muscle performance test reflect the torque profile. An example of the NIRS response during the fatigue resistance section of the muscle performance test is displayed in Figure 7.0. Further work is clearly warranted in developing the links between these two measures.
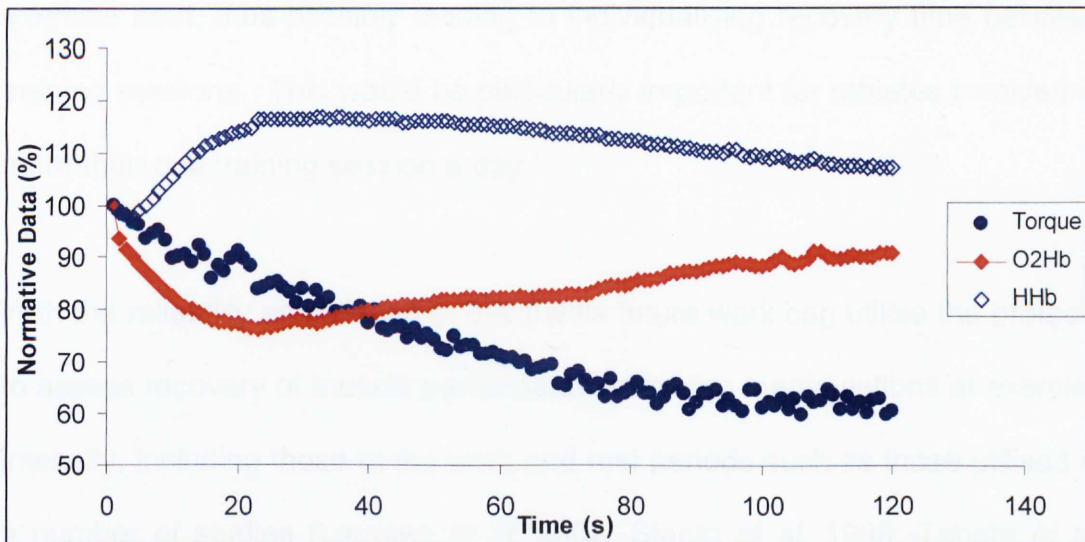
Figure 7.0 Muscle NIRS response during a fatigue trial. At the beginning of the trial there is a steep reduction in oxyhaemoglobin (O2Hb) with a concomitant rise in deoxyhaemoglobin (HHb). This response is reversed during the trial as torque output stabilises. (Unpublished data M.Morris)

The timescale for recovery in muscle performance responses in the 10 subjects tested in Chapter Six highlights the potential of using electrical stimulation protocol to monitor recovery of muscle performance following specific training bouts. Recovery of these parameters was still depressed 1 hr following the intermittent protocol, despite the same total work being completed, and thus could highlight the improved muscular adaptations often reported following intermittent training compared to those of lower, more prolonged training intensity (Burgomaster et al. 2008). However, how these acute alterations in muscle performance would effect subsequent performance has yet to be determined. Whether the full recovery of the muscle contractile measures is required to maintain optimal performance in subsequent bouts requires further investigation. The use of the protocol to measure recovery from a training session could then be a useful tool to report when muscle contractile characteristics have recovered from the preceding

exercise bout, thus possibly leading to individualising recovery time between training sessions. This would be particularly important for athletes involved in more than one training session a day.

With the reliability established in this thesis future work can utilise the protocol to assess recovery of muscle performance following manipulations of exercise intensity, including those of the work and rest periods such as those utilised in a number of studies (Laursen *et al.* 2002; Stepto *et al.* 1999; Tabata *et al.* 1996) to assess the acute effects of such interventions on *in vivo* muscle performance. It could also be utilised to assess the effects of various cadence strategies and possibly nutritional interventions to assess their impact on muscular performance.

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