

2002

## Changes in muscle function and motorneuron excitability of the triceps surae following a bout of fatiguing eccentric exercise

Mikala Pougault  
*Edith Cowan University*

Follow this and additional works at: [https://ro.ecu.edu.au/theses\\_hons](https://ro.ecu.edu.au/theses_hons)



Part of the [Exercise Science Commons](#), and the [Sports Sciences Commons](#)

---

### Recommended Citation

Pougault, M. (2002). *Changes in muscle function and motorneuron excitability of the triceps surae following a bout of fatiguing eccentric exercise*. [https://ro.ecu.edu.au/theses\\_hons/571](https://ro.ecu.edu.au/theses_hons/571)

This Thesis is posted at Research Online.  
[https://ro.ecu.edu.au/theses\\_hons/571](https://ro.ecu.edu.au/theses_hons/571)

# Edith Cowan University

## Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study.

The University does not authorize you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following:

- Copyright owners are entitled to take legal action against persons who infringe their copyright.
- A reproduction of material that is protected by copyright may be a copyright infringement. Where the reproduction of such material is done without attribution of authorship, with false attribution of authorship or the authorship is treated in a derogatory manner, this may be a breach of the author's moral rights contained in Part IX of the Copyright Act 1968 (Cth).
- Courts have the power to impose a wide range of civil and criminal sanctions for infringement of copyright, infringement of moral rights and other offences under the Copyright Act 1968 (Cth). Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

**CHANGES IN MUSCLE FUNCTION AND MOTORNEURON  
EXCITABILITY OF THE TRICEPS SURAE FOLLOWING A  
BOUT OF FATIGUING ECCENTRIC EXERCISE**

By

Mikala Pognault

Thesis submission for partial fulfilment for the award of  
Bachelor of Science (Sports Science) with Honours

Faculty of Communications, Health and Science  
EDITH COWAN UNIVERSITY

Principal Supervisor: Dr Paul Sacco  
Associate Supervisor: Carmel Nottle

Date of submission: 11 December 2002

## USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.

## ABSTRACT

A reduction in capacity of the neuromuscular system associated with exercise can occur from a wide range of physiological and psychological factors. Many researchers have investigated neural activation during exercise, or the effects of muscle damage associated with eccentric exercise, but few have studied the prolonged effects of a bout of eccentric exercise on strength and motoneuron excitability. Eleven male and female subjects (aged 20-43 years) were tested to determine the effects of a fatiguing bout of eccentric exercise upon maximal isometric plantarflexion strength, motoneuron excitability, and neural activation of the soleus (SOL) and medial gastrocnemius (MG). The exercise consisted of two hours on a calf raise machine, the only the right leg performing eccentric repetitions, with three sets of 60 repetitions at 60% of the concentric one repetition maximum (1RM).

Hoffman reflex (H-reflex), evoked responses, maximum voluntary contraction (MVC) torque, voluntary root mean squared electromyography (rmsEMG), Creatine Kinase (CK), and the Achilles tendon reflex (T-reflex) were tested immediately prior to, immediately post, and 1, 24, 48 and 72 hours post exercise. Results indicated that there were significant ( $p < 0.05$ ) decreases of 18% and 23% in MVC torque and SOL rmsEMG respectively following the fatiguing protocol. There were also significant declines of 31% in the SOL H-reflex, 25% in the SOL  $H_{max}:M_{max}$  (the ratio of the maximum H-reflex to the maximum M-response), as well as a 21% decline in the amplitude of the evoked twitch. There were no significant decreases in the M-response or T-reflex, or in any of the variables of the control leg, following the exercise bout.

The reduced voluntary torque and EMG suggests that the force loss was due to a decreased neural drive. The decline in the H-reflex following exercise indicates a reduction in the excitability of the  $\alpha$ -motoneuron pool (since altered M-waves suggest no impairment in neuromuscular propagation). The change in strength may in part be due to alterations in spinal excitability, but other factors must also contribute since the correlation between the two (although significant) is relatively

weak ( $r^2 = 0.2$ ). The lack of change in the T-reflex may suggest that, with the combined effect of a decrease in spinal excitability and increase in spindle responsiveness and/or muscle compliance, which in part compensate for the decline in  $\alpha$ -motoneuron excitability, the resultant net change was zero. Result suggests that alterations in motor drive associated with fatiguing eccentric exercise probably represent a combination of the modulatory effects of a number of inputs (both excitatory and inhibitory) to the  $\alpha$ -motoneuron.

## DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief:

- (i) incorporate without acknowledgment any material previously submitted for a diploma or degree in any institution of higher education;
- (ii) contain any material previously published or written by another person except where due reference is made in the text; or
- (iii) contain any defamatory material.

Signature..

Date...15...2...03.....

## ACKNOWLEDGMENTS

First, and foremost, to my family - you have been my typist, editor, money bank, pit crew, hotel, and sanity (or is that insanity?) for the past four years. I especially would never have made it through this last year without you. Words can't describe how grateful I am for the sacrifices you have made throughout the year.

To my crazy group of friends, especially my fellow honours buddy Sasha, Geisha partner Kinga, and B1 Emily, thank you for your friendship, support, debauchery, insanity, and most of all laughter. I can vaguely remember what you guys look like!

A big thank you to my supervisor Dr Paul Sacco, your input and guidance this year has been irreplaceable (when I could actually pin you down to read my work!). And to my Co-supervisor Carmel, my AMLAB Guru, testing and data collection would have been an absolute shambles if it weren't for your endless help. So thank you for putting up with all of my questions!

Thanks (or good riddance?) to the other postgrads, Lynda, Naomi, Nick, Michael, Dale (aka. Petal), Cherina, and Andrew. You guys have been made it a hilarious and enjoyable environment to work in and made the long hours at uni bearable. Thank you to the sports science staff for your assistance throughout the year. A special thanks to Mike Newton for your assistance with the equipment and protocol, and Nadija Vrdoljak for your technical assistance, chocolate eclairs and grappa.

Finally, a big thanks to all of my subjects, not only did you endure the long and taxing exercise protocol, but had to put up with me zapping you as well (I promise I only enjoyed it a little bit). Thank you for all your time and effort.



## TABLE OF CONTENTS

ABSTRACT .....	ii
DECLARATION .....	iv
ACKNOWLEDGMENTS .....	v
TABLE OF CONTENTS .....	vi
LIST OF FIGURES .....	ix
LIST OF TABLES .....	xi
ABBREVIATIONS AND DEFINITIONS.....	xii

### CHAPTER ONE: INTRODUCTION

1.1 Background to the study.....	1
1.2 Significance of the study.....	3
1.3 Purpose of the study.....	3
1.4 Research Questions .....	4

### CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction.....	5
2.2 Spindle reflex .....	5
2.2.1 H-reflex.....	6
2.2.2 M-response.....	8
2.2.3 T-reflex .....	8
2.3 Muscle twitch.....	9
2.4 Electromyography.....	9
2.5 Creatine Kinase.....	10
2.6 Eccentric exercise.....	11
2.7 Muscle fatigue.....	12
2.8 Recovery of muscle fatigue.....	13

### CHAPTER THREE: MATERIALS AND METHODS

3.1 Subjects .....	14
3.2 Equipment .....	14
3.3 Exercise protocol.....	15

3.4	Data collection and analysis.....	16
3.4.1	Calf raise machine .....	16
3.4.2	Testing Apparatus.....	17
3.4.3	Subject positioning for testing .....	18
3.4.5	Data acquisition software.....	19
3.4.6	Electromyography and mechanical recording .....	19
3.4.7	Reflex measurement: muscular twitch and surface action potential.....	21
3.4.8	Strength measurement.....	21
3.4.9	Blood sampling .....	22
3.5	Time course of recovery.....	22
3.6	Statistical analysis .....	23
3.7	Limitations .....	23

**CHAPTER FOUR: RESULTS**

4.1	Baseline Values and Reliability .....	25
4.2	Effects of exercise .....	26
4.2.1	Voluntary contractions.....	26
4.2.2	Evoked responses.....	28
4.2.3	Creatine Kinase .....	30
4.3	Recovery .....	30
4.3.1	Recovery of maximal voluntary contractions.....	30
4.3.2	Recovery of evoked responses.....	32
4.4	Relationships between the variables .....	33

**CHAPTER FIVE: DISCUSSION**

5.1	Changes in maximal voluntary contraction.....	36
5.2	Reduction in EMG .....	37
5.3	Neuromuscular propagation and E-C coupling.....	39
5.4	Decrease in $\alpha$ -motoneuron excitability .....	40
5.5	Tendon reflex .....	44
5.6	Relationship between the variables.....	45
5.7	Conclusions.....	45

REFERENCES .....	47
------------------	----

APPENDICES .....	54
APPENDIX A: Informed consent form .....	54
APPENDIX B: Medical Questionnaire.....	56
APPENDIX C: Physical Activity Questionnaire .....	58
APPENDIX D: Instructions for Exercise Protocol .....	60
APPENDIX E: Subject Data Sheets.....	62
APPENDIX F: Correlation Matrix.....	73
APPENDIX G: Pilot Study Data Sheets .....	75

## LIST OF FIGURES

Figure 1. A monosynaptic reflex has only one reflex synapse in its reflex arc. The synapse is between an afferent fibre and an $\alpha$ -motorneuron (Latash, 1998, p.65).....	5
Figure 2. A typical H-reflex and M-response to increasing stimulation intensity (Latash, 1998, p.67).....	6
Figure 3. Front view of the calf raise machine used in the exercise protocol (a), with a lateral view of the pin locking system (b). ....	16
Figure 4. Lateral view of subject positioning and foot strapping during testing on the DAD. ....	17
Figure 5. Location of force transducers in relation to the footplates on the DAD....	18
Figure 6. Electrode placement on the MG and SOL used during EMG analysis. ....	20
Figure 7. The SOL M-response and H-reflex response to increasing stimulus intensity. ....	25
Figure 8. Reduction in torque and EMG with a single electrical stimulus (*) and following the commencement of the MVC (+) before (a) and immediately after the exercise bout (2 hours from the commencement of exercise) (b). There was a reduction in the twitch and H-wave, but no change in the M-wave, of the evoked potentials. A reduction in the torque produced with the MVC can also be seen. ....	27
Figure 9. Reduction in torque and EMG during a MVC for the exercise period obtained immediately post set 1, post set 2, and post the final set (* p < 0.05, ** p < 0.01).....	28
Figure 10. Effect of the exercise protocol on the H-reflex (H-wave) and Tendon reflex (T-wave) across the testing period (* p < 0.05, ** p < 0.01).....	29
Figure 11. Effect of the exercise protocol on the $H_{max}:M_{max}$ ratio across the testing period, (* p < 0.05, ** p < 0.01).....	30
Figure 12. Recovery of the maximal isometric torque of the exercised leg and control leg, and evoked twitch from the baseline values (* p < 0.05, ** p < 0.01). ....	31
Figure 13. Recovery of maximal voluntary rmsEMG from baseline of the SOL and MG, the M-response can also be seen (* p < 0.05, ** p < 0.01).....	32
Figure 14. Correlation between the soleus EMG and maximal voluntary isometric torque.....	33
Figure 15. Correlation between the SOL H:M ratio and maximal voluntary isometric torque.....	34

Figure 16. Correlation between the SOL H-wave SOL T-wave..... 35

Figure 17. Summary of inputs and outputs to  $\alpha$ -motorneurons. The solid circles are inhibitory, and the dotted curved region at the pre-motorneuronal terminals denotes presynaptic inhibition acting selectively on the afferent paths to the motorneurons (Gandevia, 2001, p.1735). ..... 41

Figure 18. Summary of the possible routes for peripheral inputs to change the firing rates of motorneurons in fatigue. The effects of tendon organs are not included. Panel one depicts reflex facilitation of motorneurons exerted through the action of group III and IV afferents on the fusimotor-muscle spindle system. Panel two depicts direct reflex inhibition of motorneurons by group III and IV afferents. Panel three depicts disfacilitation of motorneurons produced by the reduction in firing of muscle spindle endings with fatigue. Panel four depicts group III and IV afferents acting via supraspinal drives and shows their complex spinal actions involving both presynaptic and polysynaptic actions (Gandevia, 2001, p.1755). .... 42

## LIST OF TABLES

Table 1. Settings, Sampling Rates, Scaling Factors, Channel Gains and Storage Factors for Data Collection with Amlab of the EMG and Torque Data for the Evoked Reflex and MVC Protocols.....	20
Table 2 Time Schedule for the Test Protocol .....	22
Table 3. Baseline Descriptive Statistics for the Average of Three Trials of the Dependent Variables of 11 subjects, and the Coefficient of Variation (CV) results .....	26

## ABBREVIATIONS AND DEFINITIONS

CK	Creatine Kinase
CNS	central nervous system
EMG	electromyogram
H:M	$H_{max}:M_{max}$ ratio
mA	milliamp
MG	medial gastrocnemius
MN	motorneuron
ms	millisecond
MSR	monosynaptic reflex
MU	motor unit
mV	millivolt
MVC	maximal voluntary contraction
NM	neuromuscular
Nm	Newton meter
NMJ	neuromuscular junction
recEMG	rectified electromyogram
rmsEMG	root mean squared electromyogram
IRM	one repetition maximum
ROM	range of movement
SOL	soleus
TS	triceps surae
V	volt
Eccentric Reflex	Contraction during which the muscle lengthens (Enoka, 1994). A voluntary muscle contraction induced by external stimulus (Latash, 1998).
Torque	The rotary effect of a force; the product of force and the moment arm (Enoka, 1994).

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background to the study

Few problems in motor control have been more extensively studied than neuromuscular (NM) fatigue. Muscular fatigue can be defined as a reduction in force generating capacity of the neuromuscular system that occurs during sustained activity, and is often used to denote an acute impairment of performance (Bigland-Ritchie & Woods, 1984). The cause of muscle fatigue has long been the subject of controversy as it is a complex phenomenon and may involve factors at many different levels contributing to force loss and therefore performance decrement.

Failure anywhere along the pathway involved in muscle activity, from the central nervous system (CNS) to cross-bridge cycling, could result in a loss of force output from the muscle (Binder-Macleod & Snyder-Mackler, 1993). The potential sites of failure can be divided into three general categories: those which lie within the CNS, those concerned with neural transmission from CNS to muscle, and those within the individual muscle fibres. Peripheral fatigue - failure of peripheral electrical propagation or contractile mechanisms - has been widely studied (Bigland-Ritchie, Johansson, Lippold, & Woods, 1983; Davis, 1995; Hakkinen, 1995; Ingalls, Warren, Williams, Ward, & Armstrong, 1998; Jones, 1981; Lepers, Hausswirth, Maffiuletti, Brisswalter, & van Hoecke, 2000; Newham, Jones, & Clarkson, 1987; Stephens & Taylor, 1972). Central fatigue - insufficient activation of the motoneuron (MN) - has been studied much less, partly because of the complexity of the central nervous system and partly because of technical difficulties (Grimby, Hannerz, Borg, & Hedman, 1981).

While it is generally agreed that much of the force loss results from contractile failure of the muscle fibres, it may result from failure of peripheral



electrical transmission, or from central fatigue (Bigland-Ritchie, Johansson, Lippold, & Woods, 1983; Stephens & Taylor, 1972). Impairment of muscle performance is not necessarily the limiting factor in force production from a fatigued muscle. Under some conditions, altered neural drive can contribute to muscle fatigue since it may be insufficient to generate the full force which it is capable of (Gandevia, Allen, Butler, & Taylor, 1996). These changes may involve altered descending supraspinal drive, changes resulting from influence of segmental spinal reflexes, and changes in recruitment patterns of  $\alpha$ -motoneurons (Latash, 1998).

Volitional and electrical tests are often used to quantify muscle fatigue (Binder-Macleod & Snyder-Mackler, 1993). Electromyography (EMG) and percutaneous electrical muscle stimulation (EMS) are two experimental techniques that have been frequently used to study muscle activation during a maximal isometric voluntary contraction (MVC) as well as the location and mechanisms of NM fatigue. In combination with EMG, additional force induced by superimposed EMS during an MVC has been used to assist in identification of central and peripheral mechanisms of fatigue (Bentley, Smith, Davie, & Zhou, 2000). To determine whether fatigue results from declining activation by the central nervous system, the rate of force loss during a MVC is compared with that from maximal nerve stimulation. If the force falls more quickly during voluntary activity and can be restored by nerve stimulation, some fatigue is said to be 'central': if not, it must have resulted from failure at some site distal to the point of stimulation and is termed 'peripheral fatigue'.

The loss of voluntary EMG activity can result from a decrease in  $\alpha$ -motoneuron excitability. As H-reflex amplitudes are an indirect measure of the  $\alpha$ -motoneuron excitability, they can then reflect the net excitability and inhibitory influences in the  $\alpha$ -motoneuron pool. Any change in the input to  $\alpha$ -motoneurons potentially has the ability to alter their muscle output, therefore the H-reflex is a useful tool for investigating muscular fatigue (Leonard et al., 1994).

Acute high intensity or prolonged duration exercise generally induces the development of fatigue that has detrimental effects on performance. Most studies of motoneuronal fatigue have been with static contractions (Gravel, Belanger, & Richards, 1987; Kirsch & Rymer, 1987), fewer studies with dynamic contractions (Hakkinen, 1993; Pinniger, Nordlund, Steele, & Cresswell, 2001), and even fewer eccentric contractions. In comparison with concentric and isometric modes of

exercise, eccentric contractions (also referred to as negative repetitions) are believed to induce a larger impairment of force-generating capacity, longer lasting changes in EMG signal, as well as morphological and histochemical changes (Kroon & Naeije, 1991). It has been reported that the residual effects of fatigue from a previous eccentric exercise bout may disrupt exercise performance during subsequent training sessions (Bentley et al., 2000; Hamlin & Quigley, 2001b; Michaut, Pousson, Babault, & Van Hoecke, 2002). The effects of fatigue induced by exercise with eccentric contractions have been observed to last from one hour (Fowles, Sale, & MacDougall, 2000), up to 48 hours post-exercise (Hamlin & Quigley, 2001a; Smith et al., 1994). Negative repetitions can lead to a high force load on the muscle, and are commonly used in athletic training. The effect of a high work load session on muscle has been found to be a primary concern among professional and recreational athletes who wish to simultaneously develop their endurance capacity and muscle strength (Bentley et al., 2000).

## **1.2 Significance of the study**

Despite the large volume of literature relating to muscle fatigue, the effect and recovery of the strength and motorneuron excitability of the triceps surae after a prolonged eccentric exercise protocol has yet to be investigated. Furthermore, the effects of fatigue are often measured during and immediately post the fatiguing protocol, but not for a prolonged recovery period. The mechanisms of muscle fatigue following eccentric exercise are not entirely understood and therefore warrant further investigation, particularly muscle activation. A greater understanding of the mechanisms associated with decrements in muscle function following eccentric exercise will be useful when considering recovery in exercise programming.

## **1.3 Purpose of the study**

The purpose of this study was to examine the characteristics of and time course of changes in muscle function and MN excitability induced by a bout of

eccentric exercise of the lower leg. It was also to identify any relationships between MN excitability and voluntary force production following an exercise bout.

#### **1.4 Research Questions**

The research addressed four main questions:

1. What is the time course for changes in voluntary strength and EMG parameters during recovery following an eccentric exercise protocol?
2. What is the time course for changes in the evoked potentials during recovery following an eccentric exercise protocol?
3. What are the possible mechanisms for the changes in strength, EMG and evoked potentials following the exercise protocol?
4. Is there a relationship between changes in voluntary strength, EMG and evoked potentials following an eccentric exercise protocol?

## CHAPTER TWO

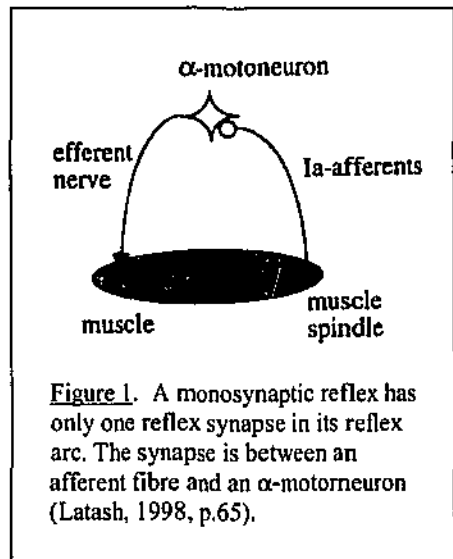
### LITERATURE REVIEW

#### 2.1 Introduction

In order to understand the research questions more clearly there are areas of the study that need to be outlined with regard to muscle fatigue. These areas are spindle reflexes, maximal voluntary strength, voluntary EMG, muscle twitch, Creatine Kinase, eccentric exercise, and recovery from muscle fatigue.

#### 2.2 Spindle reflex

A monosynaptic reflex (MSR) originates from primary spindle endings and makes only one connection (synapse) with  $\alpha$ -motoneurons of the muscle that houses the spindle (as shown in Figure 1). The fibres travel from the muscle spindle to the spinal cord and make a monosynaptic connection with the  $\alpha$ -motoneurons innervating the muscle (Latash, 1998).



In the early 1940's Renshaw (1940) (cited in Crone, Hultborn, Mazieres, Morin, Nielsen, & Pierrot-Deseilligny, 1990) introduced the MSR as a tool for investigating excitability changes in the MN pool. When used as a test reflex it allows one to

assess the effect on the MN pool of conditioning volleys in sensory afferents or descending tracts. When MNs are facilitated, the size of the test reflex increases as more MNs are recruited from the subliminal fringe by the test Ia volley, the reverse occurring with inhibition (Crone et al., 1990). One of the most common monosynaptic reflexes used in research is the H-reflex.

**2.2.1 H-reflex**

The biggest fibres within a muscle nerve are Ia afferents that originate from the muscle spindles, and are considered to have the lowest threshold to electrical stimulation. Electrical stimulation of the Ia afferents induces the monosynaptic Hoffmann reflex (H-reflex) and has been used as a tool to assess motoneuronal excitability (Bulbulian & Darabos, 1986; Garrett & Caulfield, 2001; Hoffman & Kocaja, 1995). It has also been used to investigate the modulatory changes occurring at the level of the MN pool, as well as presynaptic inhibition acting on the Ia terminals (Butler, Yue, & Darling, 1993; Crenna & Frigo, 1987; Ellrich, Steffens, Treede, & Schomburg, 1998).

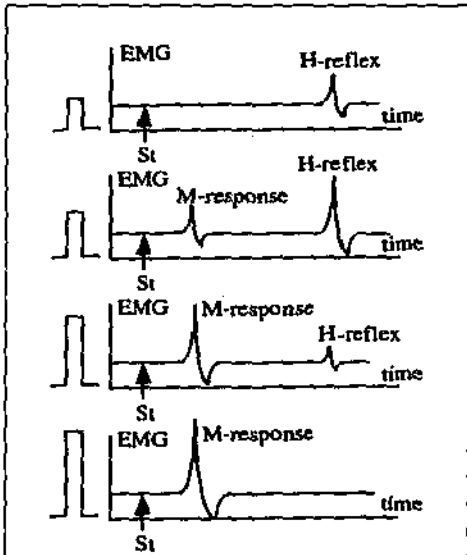


Figure 2. A typical H-reflex and M-response to increasing stimulation intensity (Latash, 1998, p.67).

The H-reflex of the lower leg is evoked by applying weak electrical stimulation of the lowest threshold to muscle spindle afferents in the tibial nerve at the knee, and has a latency of approximately 30ms. When the stimulation intensity increases the amplitude of the H-reflex increases as more Ia afferents are activated, and at some point the stimulus will induce action potentials in the axons of the  $\alpha$ -motoneurons (Latash, 1998).

A further increase in stimulus intensity will generate action potentials in more MNs, and the response (EMG and force) will be larger. For this reason the H-reflex is used as a test of the level of excitability of the motor neuron pool - the response of the H-reflex and M-response to increasing stimulation intensity can be seen in Figure 2. The bigger the response, the greater the number of motor units that have contributed to the response, because of a higher level of excitability in the motor neuron pool (Enoka, 1994). A more distal stimulation lengthens the onset latency of the H-reflex, and it is facilitated by voluntary contraction of the test muscle and inhibited by voluntary contraction of antagonist muscles. The H-reflex is also used clinically to test the function of the peripheral nerve and dorsal and ventral roots (Ellrich et al., 1998).

Alpha MNs receive monosynaptic and polysynaptic input from sensorimotor cortical projections, brain stem nuclei, and type Ia, Ib, II, III and IV sensory afferents, therefore the H-reflex amplitude reflects the net excitability and inhibitory influences in the  $\alpha$ -motorneuron pool (Leonard et al., 1994). It has been suggested that the motorneuronal excitability is affected by several factors, which can be categorised as pre- and post-synaptic (Enoka, 1994). Pre-synaptic factors are the extrinsic properties of a MN (for example, the number of synaptic terminals per MN from a given input system, and spatial distribution of synaptic terminals onto a MN). The post-synaptic factors are the intrinsic properties of a MN (eg. the total membrane area, electronic architecture of the MN which depends on the cell anatomy, the membrane time constant, and so on) (Funase, Imanaka, & Nishihira, 1994).

H-reflex amplitudes decrease during muscle fatigue (Bigland-Ritchie, Jones, & Woods, 1979; Garland & McComas, 1990; Ross, Leveritt, & Riek, 2001). Garland et al. (1990) found the soleus (SOL) H-reflex was significantly reduced and concluded that it was a result of the decreased excitability of the MN pool. Ross et al. (2001) concluded that a number of possibilities are related to spinal changes with muscle fatigue including supraspinal failure, segmental afferent inhibition, and depression of the MN excitability.

### 2.2.2 M-response

After the Group Ia afferents, the class of axons with the next largest diameter are the alpha axons, and they are recruited at a higher stimulation intensity than the Ia afferents (Figure 2). When action potentials are generated in the alpha axons, the motor response to electrical stimulation of the nerve is called the M-response and has a latency of about 8ms in the lower limb, depending on the distance between the stimulation and the muscle spindle (Enoka, 1994). Whereas the H-wave is the reflex discharge of the  $\alpha$ -motoneuron pool in response to the orthodromic afferent volley travelling in the large-diameter Ia fibres originating in the muscle spindles, the M-wave is a muscle response to direct activation of the axons of the same pool. It is elicited experimentally to probe the integrity of the circuit between the site of the stimulus (muscle nerve) and the site of the recording (usually the muscle EMG); that is, it tests the integrity of the NM propagation and can, under certain conditions, decrease during muscle fatigue (Bigland-Ritchie, 1981a).

While the maximal M-wave ( $M_{max}$ ) is elicited by supramaximal nerve stimulation and is the electrical counterpart of the activation of all motor units of the pool, the maximal H-reflex ( $H_{max}$ ) is elicited by submaximal nerve stimulation (Maffiuletti et al., 2001). The ratio of maximal H-reflex amplitude to maximal M-response ( $H_{max}:M_{max}$  or H:M) is thought to represent the number of MNs recruited through the MSR as a proportion of the MN pool (Garrett & Caulfield, 2001).

### 2.2.3 T-reflex

The tendon reflex (T-reflex) is a monosynaptic reflex induced by a quick muscle stretch induced by tapping on the muscle tendon. Muscle spindles are sensitive to muscle length and velocity and therefore a quick muscle stretch will lead to synchronised firing. The action potentials travel along the Ia afferents to the spinal cord and induce a reflex response (T-wave) of  $\alpha$ -motoneurons leading to a twitch of the muscle (reviewed by Latash, 1998). The reported response of the T-reflex to muscle fatigue has been varied (Avela, Kyrolainen, & Komi, 1999; Enoka, Hutton, & Eldred, 1980).

### **2.3 Muscle twitch**

The quantal output of a motor unit (MU) is a twitch. A twitch represents the force-time response of muscle to a single input and can be characterised by three measurements: the contraction time from force onset to peak force (time to peak or TTP), the magnitude of the peak force (twitch peak torque or TPT), and the time it takes for the force to decline to one half of its peak value (half relaxation time or HRT). Contraction time is used as a measure of the speed of the contractile machinery (Enoka, 1994).

Localised muscle fatigue has been shown to influence the electrical and mechanical properties of the muscle fibre of the active MUs, it is characterised not only by loss of force but also by a slowing of the contraction speed (Fowles et al., 2000; Fuglevand, Zackowski, Huey, & Enoka, 1993). With fatigue the amplitude of the action potentials of the MUs can decrease, the duration increase, the amplitude of the mechanical twitch reduce, and there can also be a prolongation of the relaxation process (Esposito, Orizio, & Veicsteinas, 1998; Smith et al., 1994). Fowles et al. (2000) found a decrease in contractile force up to one hour following repeated passive stretching and concluded that the excitation frequencies required to maintain a given level of muscular activation were directly proportional to the speed of contraction. Therefore, the physiological response to any change in electrical excitation depends on simultaneous changes in muscle mechanics, and loss of force may not necessarily result from a decrease in electrical activity (Bigland-Ritchie, 1981b).

### **2.4 Electromyography**

Electromyography (EMG) is a method of registration of compound action potentials generated by muscle fibres (Latash, 1998). The most common approach to measuring EMG is to place an electrode near an excitable membrane and record the action potentials as they pass the electrode, with the action potential being recorded as a voltage-time event. Following an exercise bout, if the drop in force is accompanied by a parallel decline in electrical activity, fatigue is attributed to failure of excitation -



but if the electrical activity is undiminished the failure is attributed to events within the muscle (Bigland-Ritchie, 1981a).

There is commonly a reduction in voluntary EMG following electrically induced or voluntary fatigue (Bentley et al., 2000; Bigland-Ritchie, Johansson, Lippold, & Woods, 1983; Fowles et al., 2000; Fuglevand et al., 1993). Bigland-Ritchie, Johansson, Lippold et al. (1983) showed a 40% decline in voluntary EMG following a sustained MVC, and concluded that the loss of force may have resulted from inadequate muscle activation in addition to failure of its contractile mechanism. The origin of the decline in motor unit activation is in part reflexively dependent on afferent signals from the contracting muscle. This decline may be advantageous in that it helps to protect peripheral NM structures from excessive exhaustion and prevent impulse frequencies higher than those needed for a full tetanic activation of the fatiguing muscle fibres (Avela et al., 1999), for example, when contractile properties are slowing.

## **2.5 Creatine Kinase**

Increased serum levels of Creatine Kinase (CK) is commonly used as an indirect marker of the microtrauma which can occur in response to unaccustomed exercise or an increase in the volume or intensity of exercise (Clarkson, Byrnes, McCormick, Turcotte, & White, 1986; Newham, Jones, & Edwards, 1983). The level of CK and time course of recovery depends on the type and intensity of the exercise bout. It has been shown that CK increases significantly following moderate and high intensity exercise, as well as eccentric exercise bouts (Clarkson et al., 1986; Dolezal, Potteiger, Jacobsen, & Benedict, 2000; Newham et al., 1987; Newham, Jones et al., 1983; Raastad & Hallen, 2000; Smith et al., 1994). It has been reported that there is a larger increase in CK with high intensity exercise than with moderate intensity exercise (Raastad & Hallen, 2000), and depending on the exercise prescription, CK peak can occur anywhere from six hours to five days (Clarkson, Kroll, & McBride, 1980; Newham, Mills, Quigley, & Edwards, 1983; Raastad & Hallen, 2000).

## 2.6 Eccentric exercise

The particular site, or combination of sites, that contribute to reduction in force generating capacity is likely to depend on the type and intensity of the muscular activity causing the fatigue. There is emerging evidence that the activation of a motor unit pool may vary with the relative magnitude of the muscle and load torques. When the muscle torque is less than the load torque, the active muscle lengthens in an eccentric contraction. It has been reported that it is difficult for subjects to generate a maximal CNS drive to the motor unit pool during eccentric conditions, at least in comparison to that achieved in concentric conditions (Enoka & Stuart, 1992). As well as the specific type of fatiguing load, the magnitude of a fatigue-induced decrease in the NM performance is related to the overall volume and intensity of the session (Hakkinen, 1993). Strenuous heavy resistance continuous muscular work usually leads to momentary changes both in the maximal voluntary neural activation of the exercised muscles and in muscular strength (Hakkinen, 1993).

Impairment of force-generating capacity due to eccentric exercise is well demonstrated (Hamlin & Quigley, 2001a, 2001b; McHugh, Connolly, Eston, Gattman, & Gleim, 2001; Moritani, Oddson, & Thorstensson, 1990; Newham, Mills et al., 1983; Pearce, Sacco, Byrnes, Thickbroom, & Mastaglia, 1998), with the impairment persisting for several days or weeks (Hamlin & Quigley, 2001b; Kroon & Naeije, 1991; Michaut et al., 2002; Saxton et al., 1995; Smith et al., 1994). Strength losses after eccentric exercise have been reported to be greatest in the first 24 hours after a bout of eccentric exercise and may well be on the way to recovery, or have fully recovered, by the time that soreness develops (Hamlin & Quigley, 2001b).

The issue of whether force decrease induced by eccentric muscle actions could also be partly attributed to central fatigue is still unsettled. After voluntary eccentric exercise, Saxton et al. (1995) did not find any central fatigue following 50 maximal eccentric contractions, whereas Gibala et al. (1995) reported a 6% voluntary activation decrease using the twitch interpolation technique after eight sets of eight repetitions at 80% of one repetition maximum. Most of the studies dealing with eccentric muscle actions have primarily focused on strength recovery, while the mechanisms of the recovery of fatigue following an eccentric exercise are less discussed (Gibala,

MacDougall, Tarnopolsky, Stauber, & Elorriaga, 1995; Michaut et al., 2002; Saxton et al., 1995).

## 2.7 Muscle fatigue

Volitional activation of skeletal muscle requires proper functioning of both the CNS and peripheral NM pathways, therefore muscle fatigue may reflect the ability to achieve full voluntary muscle activation (Bigland-Ritchie, 1981b). Maximal voluntary contraction force declines with prolonged exercise and has been used as a most common index of fatigue. The drop in muscle force may be accompanied by a decrease in  $\alpha$ -motorneuron excitability and reduced frequency of firing of individual motor units (Latash, 1998). The central processes involve the activation of the motor portions of the cerebral cortex and MN pool in the ventral gray matter of the spinal cord. Peripheral activation begins with the transmission of action potentials along the peripheral motor nerve axon, continues across the neuromuscular junction (NMJ) to the muscle membrane and the transverse tubular system, and ends with the cross-bridge formation between the myosin heads and actin filaments (Stackhouse et al., 2001).

Controversy exists over whether central fatigue plays a major role in the loss of force associated with fatigue. It is often assumed that there is a complete activation of the muscle when no extra force can be elicited by electrical stimulation. However, under some conditions, there may be a failure of central motor drive which results in sub-maximal activation of the muscle (Kent-Braun & Le Blanc, 1996; Stackhouse et al., 2001). A number of studies have indicated that muscle fatigue is associated with a decrease in neural activation of motor units (Bigland-Ritchie, Johansson, Lippold, Smith, & Woods, 1983; Bigland-Ritchie & Woods, 1984; Enoka & Stuart, 1992; Hakkinen, 1993; Moritani et al., 1990). It has also been observed that during muscle fatigue changes in the corticomotor excitability occur (Gandevia et al., 1996; Sacco, Thickbroom, Byrnes, & Mastaglia, 2000), as well as a modulation of muscle activation in order to preserve force output and NM transmission (Kirsch & Rymer, 1987; Leonard et al., 1994).

## **2.8 Recovery of muscle fatigue**

Only a small number of muscle fatigue studies have followed recovery over a prolonged period. Kroon and Naeije (1991) simultaneously recorded muscle performance and the surface EMG up to 25 hours after the dynamic exercise of the human biceps brachii muscle to exhaustion. The study indicated that after the heavy dynamic exercise the recovery rate of the EMG was similar to the rate of recovery of muscle performance. A decrease in MVC up to one hour post-exercise has been reported by Fowles et al. (2000) and Fuglevand et al. (1993), while Hamlin et al. (2001b) found a 12% decrease in EMG and was still decreased at 48 hours. Smith et al. (1994) found a significant time effect of eccentric exercise on strength, and that the greatest reduction was found 48 hours after exercise, but was only represented by a 9% decrease in strength.

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 Subjects**

Subjects were recruited from the staff and student population of the School of Biomedical and Sports Science at Edith Cowan University, as well as from the friends and family of the researcher. Eleven healthy adults (four female, seven male), with a mean age, height, and weight of  $25.8 \pm 6.4$  years,  $172.7 \pm 7.94$  cm, and  $72.5 \pm 10.4$  kg respectively, participated in the study. All subjects completed an informed written consent (Appendix A), medical questionnaire (Appendix B), and physical activity questionnaire (Appendix C) prior to testing. Subjects were screened to eliminate those who: had participated in heavy resistance training in the last six months; had muscular / neurological disorders; had injuries of the lower leg in the last six months; or had been taking medications that may affect the CNS or muscle function. Approval to undertake research involving human subjects was given by the Committee for the Conduct of Ethical Research at Edith Cowan University.

#### **3.2 Equipment**

Dual Ankle Dynamometer (Ribuck Industries)

Electric Stimulator (model DS7, Digitimer)

Bipolar Stimulation Electrode (Medelec)

Microsoft Excel 2000

AMLAB Computer Software (version 2)

Surface EMG Conductive Adhesive Electrodes (Meditrace 200 Ag/AgCl, Kendall)

Conductive Gel (MES)

Modified calf raise machine (RM Sporting Supplies)  
Monark Cycle Ergometer (818E, Ergomedic)  
Spectrophotometer (Reflotron, Boehringer-Manheim)  
Creatine Kinase test strips (Reflotron, Boehringer-Manheim)  
Lancet (Boehringer-Manheim)  
Capillary tubes (Boehringer-Manheim)  
Metronome (System Maelzel)  
Tendon hammer (AMA medical products)  
Goniometer (AMA medical products)

### **3.3 Exercise protocol**

The protocol consisted of one exercise bout of 180 repetitions on a modified calf raise machine (Figure 3a). The repetitions were eccentric in nature, and had a weight load of 60% of each subjects concentric one repetition maximum (1RM). Three sets of 60 repetitions were performed, with a three minute rest time between sets. Each repetition took approximately 10 seconds to complete at a metronome governed pace, and the entire exercise bout took approximately two hours to complete. To standardise the protocol, the right leg was exercised, with the left leg as the control for all subjects.

Subjects stood under the shoulder pads on the machine with both feet in dorsiflexion, then were instructed to plantarflex through their full range of movement (ROM). The pin was secured to maintain the position of the machine while the subject obtained the position of the exercised leg for the next repetition (Figure 3b). The subject then slowly lowered to full dorsiflexion with the body weight supported on the exercised leg only. The control leg was left in a non-weight bearing position which was decided by the individual subject. Subjects were instructed to maintain correct body positioning by keeping their back straight, and their knee extended. A standard set of instructions was given prior to the commencement of testing (see Appendix D for protocol instructions to the subject).

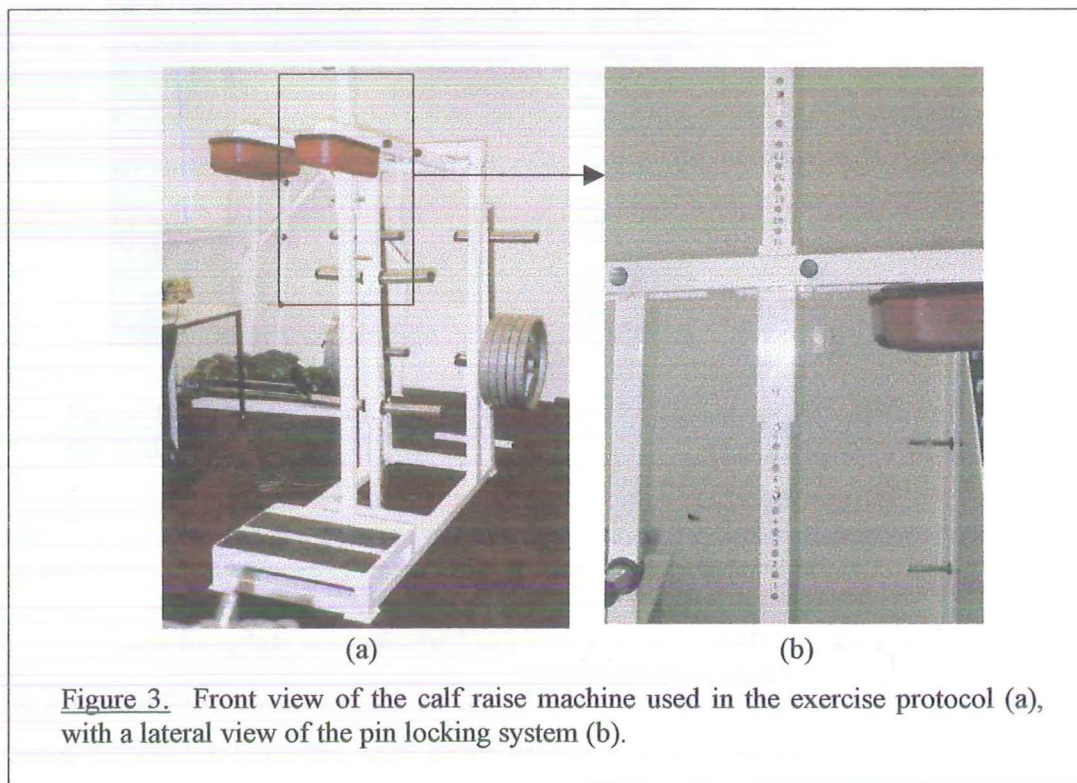
Prior to the exercise protocol the subjects performed a standardised warm up consisting of two minutes of cycling on an ergometer at 50 watts (50 revolutions per

minute x 1 kg), followed by two minutes stretching of the lower leg muscles. The subjects were asked to refrain from other exercise, stretching or massage during the course of the study.

### 3.4 Data collection and analysis

#### 3.4.1 Calf raise machine

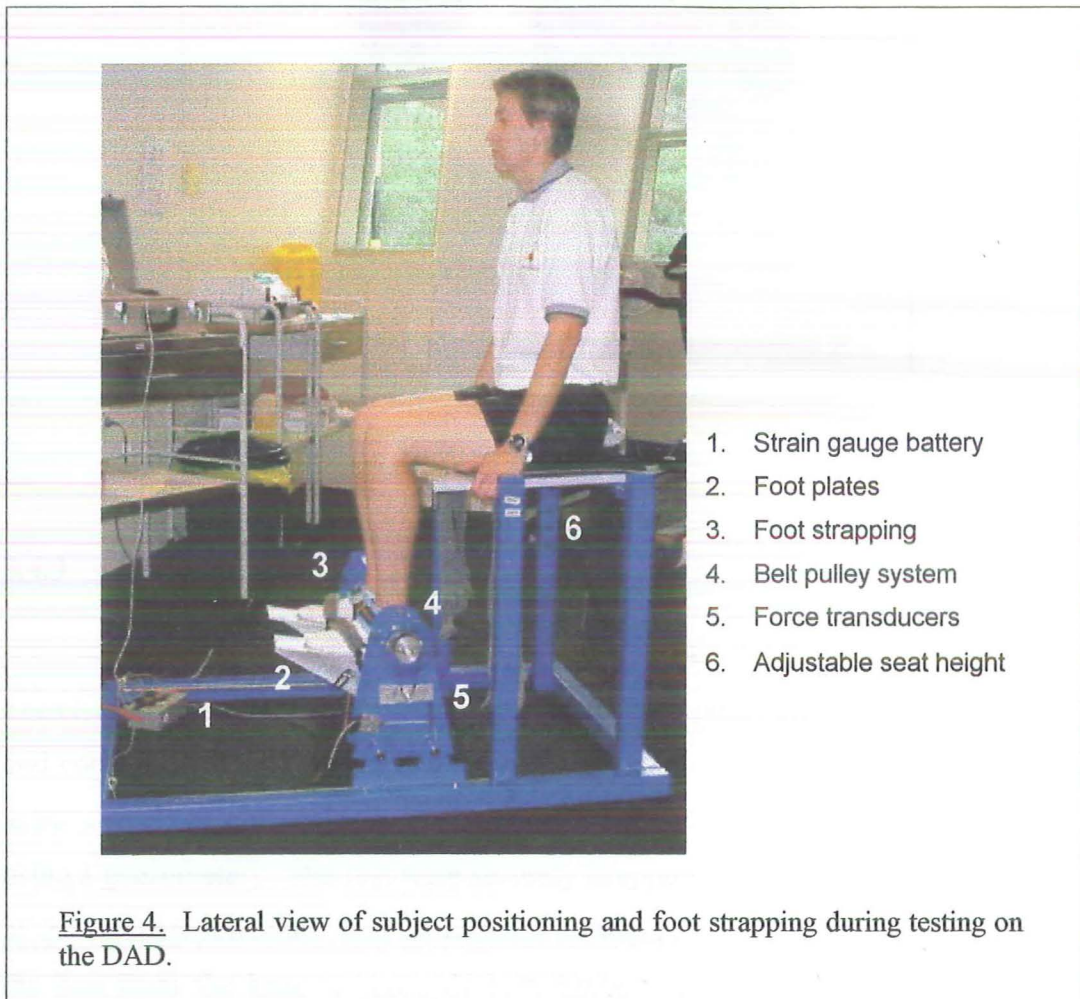
The equipment used for the exercise protocol was a modified calf raise machine (RM Sporting Supplies) used with free weights (Figure 3). It was a basic standing calf raise machine with slight modifications, such as a pin locking system to allow for heavy eccentric loading without the concentric phase of a calf raise.



**Figure 3.** Front view of the calf raise machine used in the exercise protocol (a), with a lateral view of the pin locking system (b).

### 3.4.2 Testing Apparatus

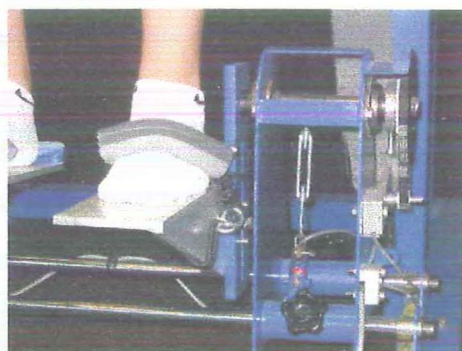
A custom built (Ribuck Industries) Dual Ankle Dynamometer (DAD) was used for the testing protocol. The DAD consisted of a base frame with a variable seat height, mounted with two footplates that could be adjusted for both plate height and distance between the two plates. A lateral view of the DAD can be seen in Figure 4.



Each footplate was attached to a rotating rod connected via a belt pulley system to a displacement transducer. Using a force transducer fixed to the rotating rod via a 5mm turnbuckle (Zenith), each footplate could be locked into position to give plantar flexion torque at variable angles. The force transducers (Radio Spares model 021-300) were foil (copper nickel alloy) uni-axial strain gauges (resistance  $120\Omega$ , Wheatstone bridge) receiving a constant DC input from a 9V battery (Figure 5). All output signals



from the strain gauges were relayed via shielded cabling to a personal computer (PC) running AMLAB software.



**Figure 5.** Location of force transducers in relation to the footplates on the DAD.

### **3.4.3 Subject positioning for testing**

The evoked reflex and tendon tap tests (section 3.4.7) were performed on the exercised leg, while MVC tests (section 3.4.8) were performed on both the exercised and control leg. For all testing on the DAD the subject sat with the trunk thigh angle at  $90^\circ$  flexion, the knee angle at  $90^\circ$  flexion, and the foot at  $10^\circ$  dorsiflexion (measured using a goniometer). The feet were securely strapped to the foot plates over the region of the extensor reticulum, and the distance between the foot plates was adjusted so that the line from the knee to ankle of both limbs was parallel to each other and was therefore perpendicular to the axis of rotation. The height of the footplate was also adjusted so the axis of rotation of the plate was aligned with the lateral malleolus. A general requirement was that the subjects were relaxed and passive throughout the tests and that the leg positions were maintained by the equipment rather than by the subject (Figure 4).

### 3.4.5 Data acquisition software

All data from the DAD was recorded, stored and analysed using AMLAB 'Windows' based software (version 2.0) and hardware (single digital signal processor, mini-rack interface, and 18 channel isolated ground card) computer application package. Signals from the DAD were sampled and viewed as a voltage change using AMLAB and the data was stored on hard disk for offline analysis. The sampling rates, scaling factor, channel gain, and storage factors for the wave recordings of the reflexes can be seen in Table 1. Conversions from volts to torque (Nm) were based on calculations determined via prior calibration procedures. Calibration involved loading each DAD footplate fixed at 10° dorsiflexion with 25lb in weights and recording the subsequent voltage reading through AMLAB. The same method of weight application was used on the Cybex 6000 isokinetic dynamometer, hence the following calculation was used to convert voltage recordings to torque values in Nm. Calibration was carried out weekly during the testing period.

$$\begin{aligned} 25\text{lb on the Cybex} &= 17.43 \text{ Nm} \\ 25\text{lb on the DAD foot plate} &= 63.44 \text{ V} \\ 63.44 / 17.43 &= 3.64 \\ \therefore 1 \text{ Nm} &= 3.64 \text{ V} \end{aligned}$$

### 3.4.6 Electromyography and mechanical recording

After careful preparation of the skin (abrasion and cleaning with alcohol) pairs of surface electrodes (Meditrace 200, Ag/AgCl) were placed on the soleus (SOL) approximately 13cm above the calcaneus and below the muscle fibres of the gastrocnemius, as well as on the gastrocnemius medial head (MG) approximately 7cm below the caput fibulae. The surface electrode pair were placed at a distance of 30mm centre to centre. Electrode placements can be seen in Figure 6. The reference electrode was placed on the bony prominence of the patella. Actual electrode positions were carefully measured for each subject to control that they were identical for each time period. EMG analysis of muscular activity was conducted during the MVC and reflex protocols. EMG signals collected during the reflex protocols (reflex EMG) were amplified, filtered, displayed, stored and analysed in raw format. EMG

signals collected during the MVC protocol (rmsEMG) were amplified, filtered, rectified, displayed, stored using AMLAB, then exported to Microsoft Excel where an average of the values collected over one second was calculated for data analysis (Table 1).

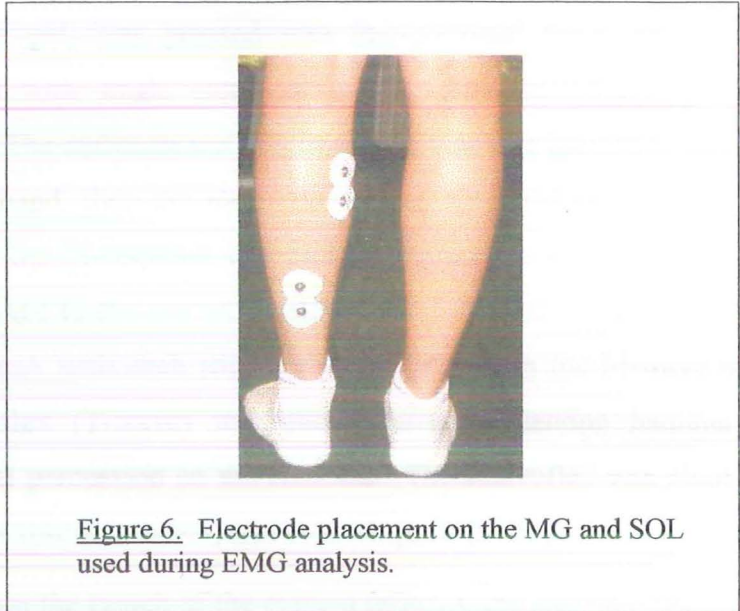


Table 1.

Settings, Sampling Rates, Scaling Factors, Channel Gains and Storage Factors for Data Collection with Amlab of the EMG and Torque Data for the Evoked Reflex and MVC Protocols

Protocols	Filtering		Sampling Rate (Hz)	Scaling Factor	Channel Gain	Storage Decimation Factor
	Low pass	High pass				
Reflex EMG	3.52	1025.16	4000	2	2000	1
rmsEMG	5.74	478.98	1000	2	4000	1
Torque						
Left gauge	-	-	1000	-245	100	5
Right gauge	-	-	1000	-295	175	5

### **3.4.7 Reflex measurement: muscular twitch and surface action potential**

Reflexes were evoked by electrical stimulation of the tibial nerve in the popliteal fossa on the exercised leg only, and were elicited using a high voltage stimulator (model DS7, Digitimer). A bipolar stimulation electrode (Medelec), consisting of two small foil pad electrodes wrapped in wet gauze and covered with conductive gel, was pressed into the popliteal fossa, and the tibial nerve was stimulated with single electrical pulses (duration 0.1ms) delivered at 10 second intervals. The optimum site of stimulation was first located by holding the stimulation probe by hand, then the electrode was manipulated until a consistent H-reflex was found and the M-response was minimal, subsequently, the stimulation electrode was firmly affixed to the site with velcro straps. The stimulus intensity was increased by 0.5 - 1.0 mA with each trial until no increases in the M-wave could be seen. The tendon reflex (T-wave) was elicited using a tendon hammer by performing a mechanical percussion on the Achilles. The test reflex was elicited eight times with 10 seconds rest in between, and an average of the eight trials was used in data analysis.

From the twitch of the evoked reflexes, the maximal twitch torque (TPT), time to peak (TTP), and half relaxation time (HRT) was measured. The TPT and TTP measurements were both taken from the initiation of the twitch torque to the point of the peak torque, and the HRT was taken after the peak torque from 90% to 45% of the recovery of the twitch (Alway, MacDougall, & Sale, 1989). Also measured were the peak-to-peak amplitudes in volts (V) of the surface action potentials of the H-reflex and Tendon reflex waves for each trial for each subject. The peak to peak amplitude of the maximum motor response ( $M_{max}$ ) was measured, and the peak H-reflex ( $H_{max}$ ) was expressed as a ratio of the  $M_{max}$  ( $H_{max}:M_{max}$  ratio).

### **3.4.8 Strength measurement**

The MVC test was performed pre-exercise, after each exercise set, and for each recovery time period. Peak torque and rmsEMG obtained during the maximal isometric contraction for the exercised and control legs were determined from the average of three trials. The subject was instructed that they were able to lift the heel off the footplate, but refrain from holding the DAD frame with their hands. Three

trials were performed with a single electrical pulse delivered towards the end of the third trial in order to determine if there was a change in the torque readings when stimulated. Subjects were encouraged verbally to exert a maximal constant effort by isometrically contracting the calf muscle into plantarflexion against the footplate for 10 seconds during the trials.

### 3.4.9 Blood sampling

Using a lancet to puncture the skin capillary blood samples were drawn from the subjects fingertip. The blood was collected in a 30mL heparinized capillary tube and analysed for blood CK using a portable spectrophotometer (Reflotron, Boehringer-Manheim) after each testing period.

### 3.5 Time course of recovery

The time course of recovery for each of the variables measured following the exercise bout was determined. Therefore, the H-reflex, tendon tap, and CK tests were performed immediately post; and 1, 24, 48, and 72 hours post exercise, while the MVC tests were also measured after each set of the exercise protocol. The schedule for the test protocols can be seen in table 2.

Table 2  
Time Schedule for the Test Protocol

Protocol	Baseline	Exercise	Hours post exercise				
			0	1	24	48	72
Exercise Protocol		*					
Evoked reflexes	*		*	*	*	*	*
Tendon tap	*		*	*	*	*	*
MVC	*	*	*	*	*	*	*
CK	*		*	*	*	*	*

### **3.6 Statistical analysis**

Statistical analysis on the data of the 19 parameters acquired during the testing period was carried out using SPSS (version 10.0) for Windows. Variables acquired from the MVC test were assessed using a 1 x 8 repeated measures factorial ANOVA, with post hoc contrasts to baseline. All other variables were assessed using a 1 x 6 repeated measures factorial ANOVA, with post hoc contrasts to baseline. Greenhouse-Geisser corrections were applied to significant analyses of variance that did not meet Mauchly's sphericity assumption, with the level of significance set at  $p < 0.05$ . A Pearson product moment correlation matrix was generated to show the degree of relationship among the variables. Descriptive statistics for the baseline values (mean  $\pm$  standard deviation) were tabulated for all variables, and the data was normalised to the baseline values and analysed for changes according to the baseline. Reproducibility data was collected during a pilot study, and from the results the coefficient of variation of repeated measures was calculated for each of the dependent variables (Norman & Streiner, 1999).

### **3.7 Limitations**

There were several limitations to the present study. Firstly, with the mean age of the subjects being  $26 \pm 6.83$ , and subjects who were resistance trained or injured were excluded, therefore, the subjects may not have been a true representation of the population. Secondly, there were two instances of equipment failure during the testing period, which meant that some data was missing for two testing time points. Thirdly, the subjects were relied upon to perform MVCs to the best of their capabilities, and were given consistent and strong encouragement by the same tester. It was also assumed that the subjects refrained from stretching and exercise within the testing period, however, it was only suggested and not enforced or monitored. Fourthly, a limiting factor in the present study was that central activation ratio was not measured, therefore the voluntary force and the maximal evokable force could not be compared. Finally, the methods themselves are not without their limitations, the electrically

stimulated contractions can be uncomfortable, and may cause inadvertent stimulation of the antagonist muscles of the lower leg.

## CHAPTER FOUR

### RESULTS

#### 4.1 Baseline Values and Reliability

Table 3 shows the baseline results obtained for this study. The mean torque produced during baseline maximal voluntary contractions were  $68.5 \pm 18.8$  Nm for the exercised (right) leg, with the control (left) leg strength being marginally lower ( $56.5 \pm 16.7$  Nm). Baseline voluntary EMG (rmsEMG) ranged from  $0.20 \pm 0.08$  mV for the control MG to  $0.33 \pm 0.13$  mV for the exercised SOL, with the SOL generally higher than the MG values. Figure 7 shows the response of the SOL evoked potentials to increasing stimulus intensity for a single individual. The amplitude of the H-wave for the SOL was larger than that of the M-wave, values of the SOL H:M ratio was almost double that of the MG  $H_{max}:M_{max}$ , and the baseline value of the peak twitch torque was  $9.2 \pm 2.7$  Nm.

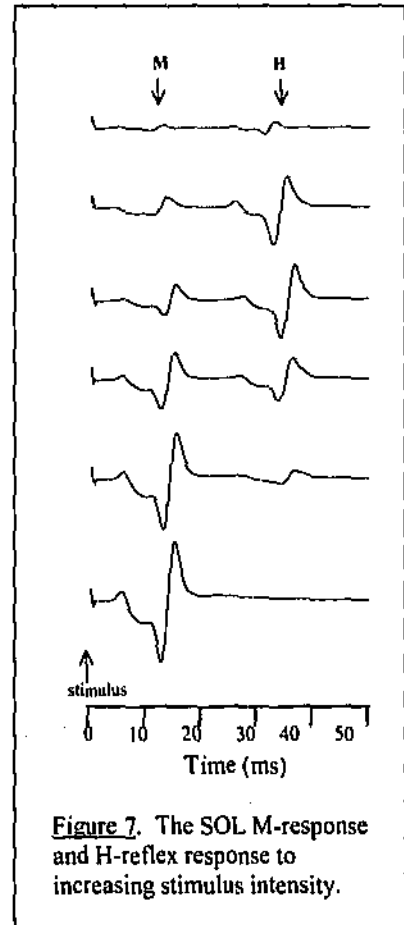


Figure 7. The SOL M-response and H-reflex response to increasing stimulus intensity.

In order to test the reproducibility of the dependant variables, a pilot study was conducted prior to testing with a sub-sample of the subjects ( $n=11$ ). Coefficient of variation of repeated measures was less than 5% for the majority of tests, with the



reproducibility ranging from 2.79% for the exercised SOL rms EMG up to 8.67% for the SOL T-wave (Table 3).

Table 3.

Baseline Descriptive Statistics for the Average of Three Trials of the Dependent Variables of 11 subjects, and the Coefficient of Variation (CV) results

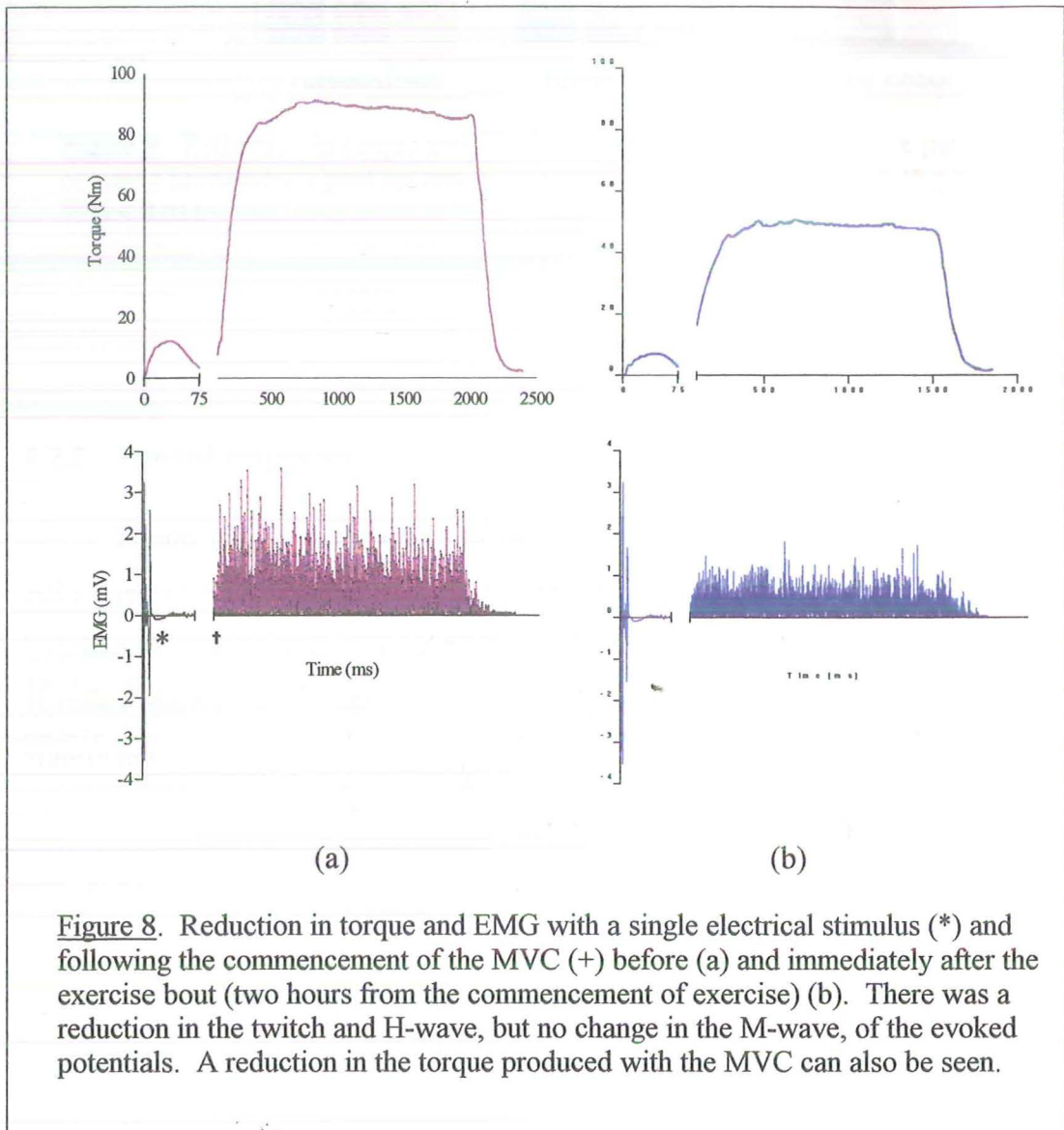
Variables	Mean	SD	CV (%)
Maximum Voluntary Torque (Nm)			
Left	56.58	16.73	5.28
Right	68.59	18.89	4.97
Maximum Voluntary EMG (mV)			
Left soleus	0.29	0.12	2.79
Left gastrocnemius	0.20	0.08	5.16
Right soleus	0.33	0.13	3.29
Right gastrocnemius	0.31	0.09	6.98
H-wave (mV)			
Soleus	3.60	1.74	5.06
Gastrocnemius	1.33	0.64	6.85
M-wave (mV)			
Soleus	8.45	2.06	3.28
Gastrocnemius	7.73	3.89	3.05
H <sub>max</sub> :M <sub>max</sub> ratio			
Soleus	0.41	0.12	5.33
Gastrocnemius	0.22	0.15	4.22
Evoked twitch			
Half relaxation time (ms)	67.00	15.87	1.38
Time to peak (ms)	129.00	6.47	2.97
Torque (Nm)	9.29	2.76	3.09
T-reflex			
Soleus amplitude (mV)	2.20	0.89	8.67
Gastrocnemius amplitude (mV)	0.82	0.42	4.16
Torque (Nm)	4.21	1.26	8.24
Creatine Kinase	124.00	53.29	

## 4.2 Effects of exercise

### 4.2.1 Voluntary contractions

All subjects showed a reduction in MVC performance over the course of three sets of the exercise protocol, there was however, a large variation in voluntary

torque loss between subjects, with strength declining to 49.24 – 88.44% of the baseline values. An example of the reduction in torque and recEMG for a single subject can be seen in Figure 8. The mean decline in MVC torque was  $82.6 \pm 10.0\%$  of the baseline ( $p = 0.003$ ) after the third set. Similarly, the decline in rmsEMG occurred post set two at  $76.2 \pm 22.1\%$  of the baseline ( $p = 0.027$ ) for the SOL and  $37.6 \pm 14.5\%$  of the baseline ( $p = 0.002$ ) for the MG. For the non-exercised leg, there were no significant changes in the torque ( $94.8 \pm 9.7\%$  after set one), SOL rmsEMG ( $76.3 \pm 23.6\%$  after set one), or MG rmsEMG ( $99 \pm 35.8\%$  after set three) over the entire testing period. The reduction in MVC torque and rmsEMG of the exercised leg following each set of the exercise protocol can be seen in Figure 9.



**Figure 8.** Reduction in torque and EMG with a single electrical stimulus (\*) and following the commencement of the MVC (+) before (a) and immediately after the exercise bout (two hours from the commencement of exercise) (b). There was a reduction in the twitch and H-wave, but no change in the M-wave, of the evoked potentials. A reduction in the torque produced with the MVC can also be seen.

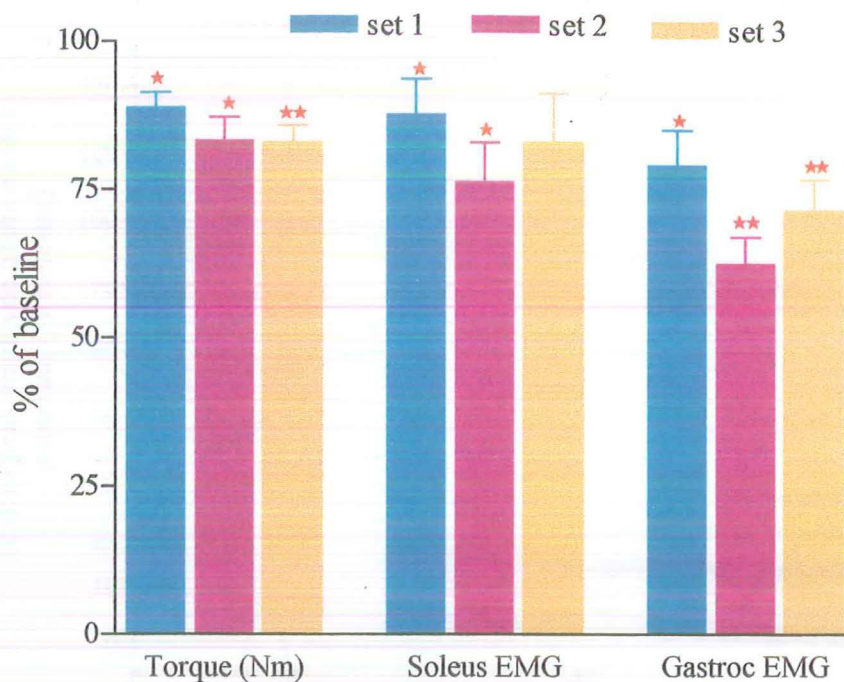


Figure 9. Reduction in torque and EMG during a MVC for the exercise period obtained immediately post set one, post set two, and post the final set (\*  $p < 0.05$ , \*\*  $p < 0.01$ ).

#### 4.2.2 Evoked responses

Figure 10 shows the effect of the exercise bout on the H-reflex and T-reflex. All subjects showed a variable reduction in the SOL H-reflex of 24.5 – 83.16% of the baseline, with a mean decline to  $68.7 \pm 31.0\%$  of the baseline ( $p = 0.015$ ). The MG H-reflex showed a similar exercise effect as the SOL, but the change was not significant.

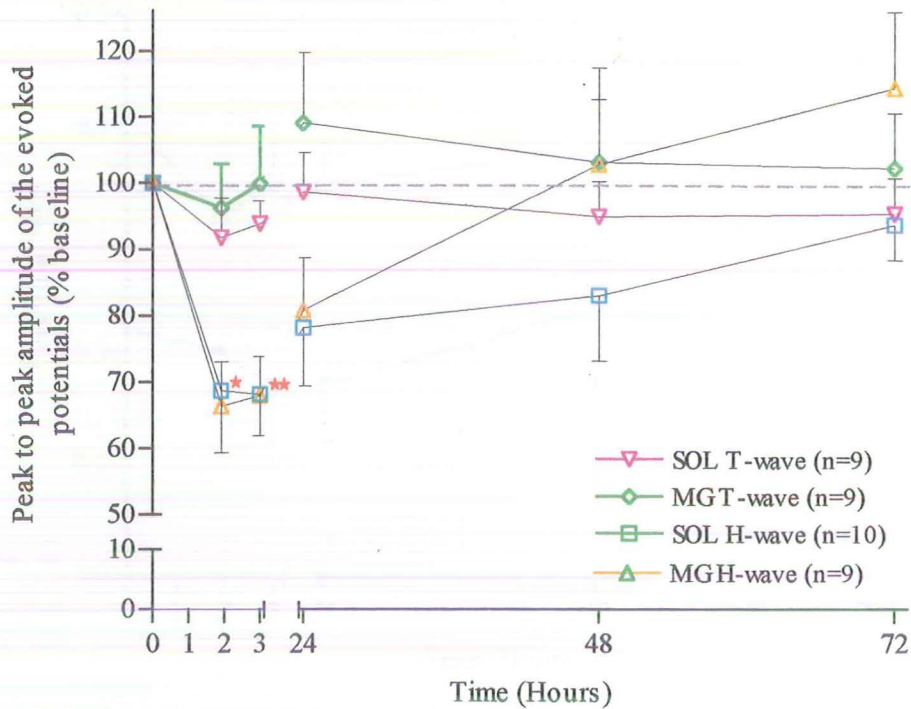
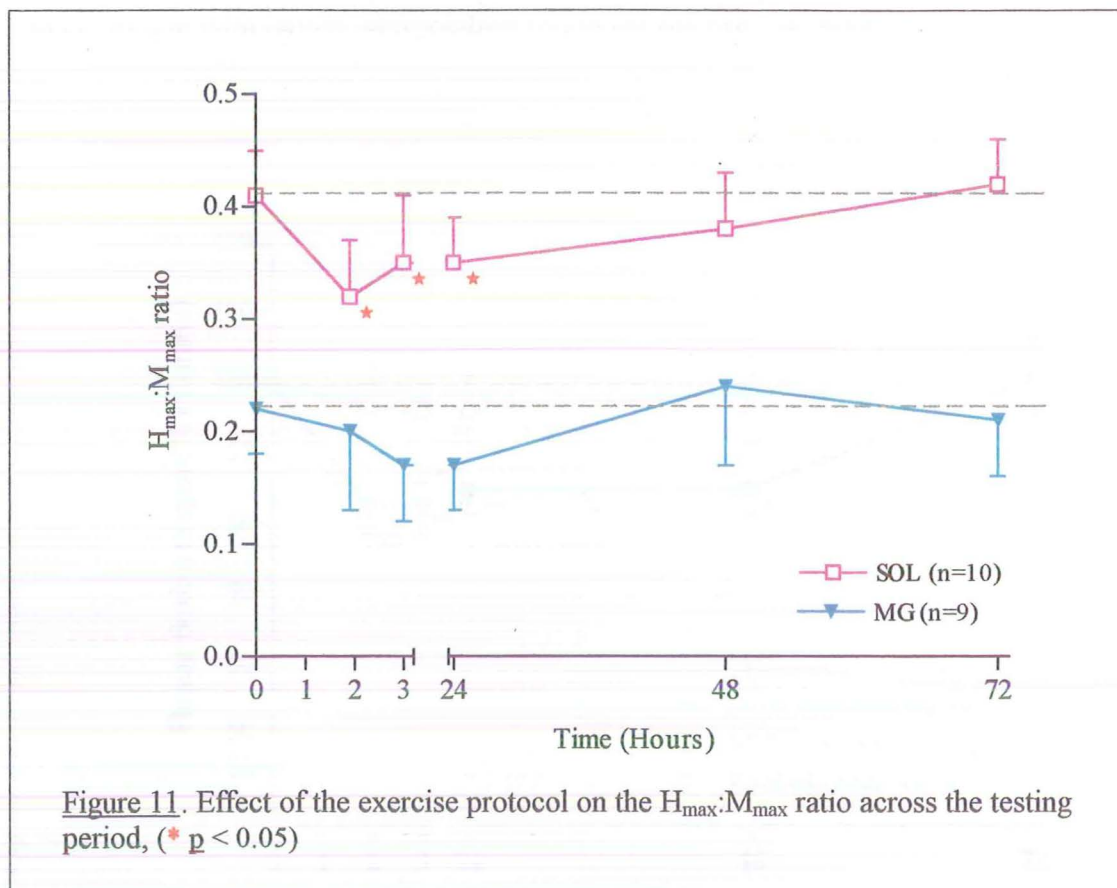


Figure 10. Effect of the exercise protocol on the H-reflex (H-wave) and Tendon reflex (T-wave) across the testing period (\*  $p < 0.05$ , \*\*  $p < 0.01$ ).

The mean decline in the SOL  $H_{max}:M_{max}$  ratio (H:M) was  $74.8 \pm 27.8$  % of the baseline ( $p = 0.01$ ), with the MG H:M showing a non-significant decline of  $24.0 \pm 35.1$ % of the baseline (Figure 11). There were no significant changes in the SOL and MG M-wave ( $86.18 \pm 14.4$ % and  $91.8 \pm 15.7$ % respectively) from the baseline values within the testing period. The amplitude of the evoked twitch showed a similar decline to the MVC torque, H-reflex and H:M immediately following the exercise bout at  $79.0 \pm 16.0$ % of baseline ( $p = 0.002$ ), this can be seen in Figure 12. There were, however, no significant changes in the HRT and TTP of the evoked twitch, or with of the variables associated with the T-reflex (T-wave) after the exercise protocol (Figure 10).



### 4.2.3 Creatine Kinase

All subjects showed an increase in CK following the exercise protocol. There was a large variation between subjects with CK increasing to 118.3 – 471.2% of the baseline values, but the average change was not significant.

## 4.3 Recovery

### 4.3.1 Recovery of maximal voluntary contractions

Figure 12 portrays the prolonged recovery of MVC torque of the exercised leg, 48 hours post-exercise it was at  $84.9 \pm 12.4\%$  of the baseline ( $p = 0.017$ ) but had

recovered by 72 hours. Although not measured, there was no observed change in the MVC torque with twitch interpolation following the exercise bout.

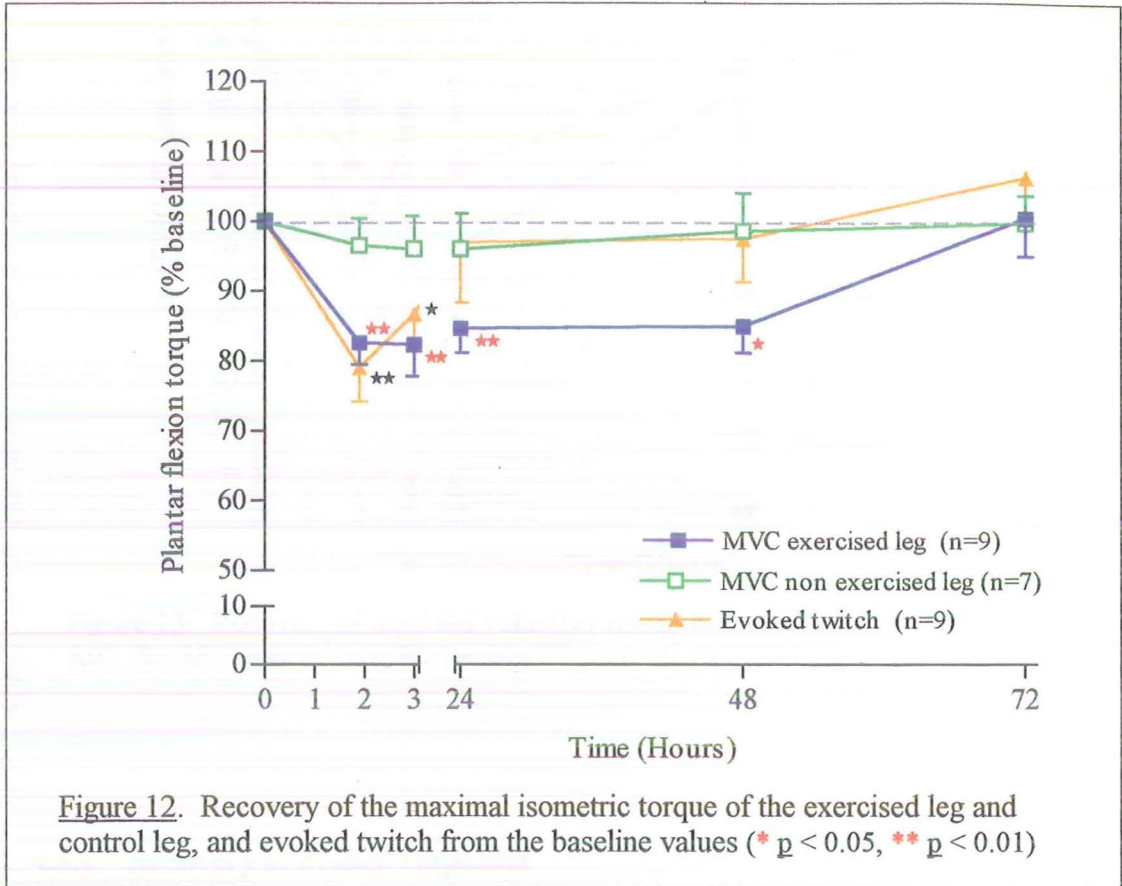
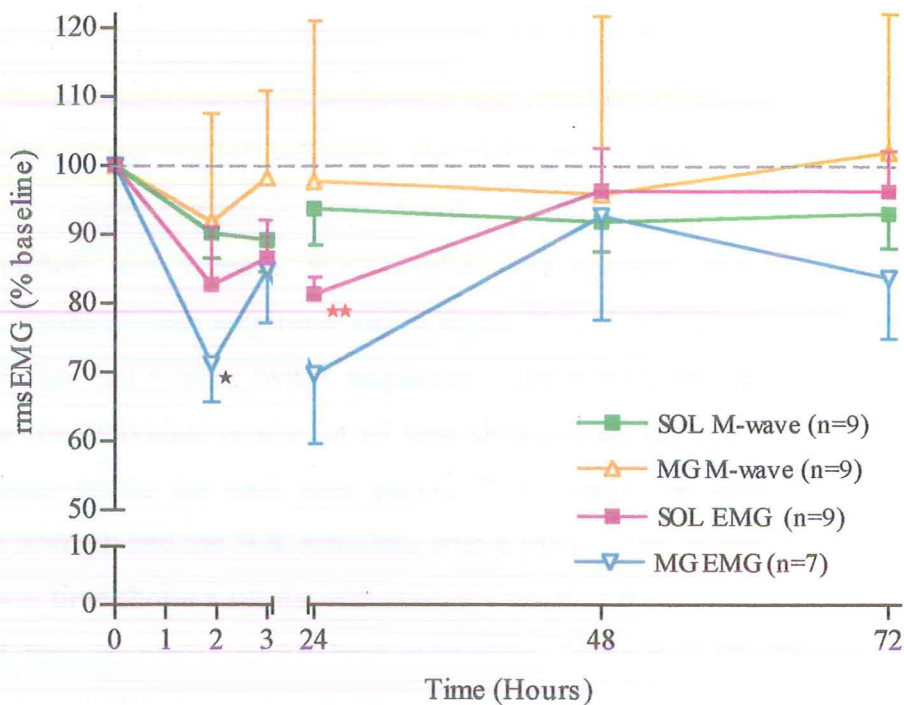


Figure 13 portrays a different pattern of recovery of the rmsEMG compared to torque with an MVC, with both SOL and MG rmsEMG recovering slightly after one hour post exercise. The SOL declined again at  $81.3 \pm 8.0\%$  of the baseline ( $p = 0.001$ ), while the MG had a larger but non-significant decrease at  $69.7 \pm 32.0\%$  of baseline at 24 hours post exercise. By 48 hours post-exercise the SOL and MG rmsEMG had again recovered to almost the pre exercise values and remained the same at 72 hours.



**Figure 13.** Recovery of maximal voluntary rmsEMG from baseline of the SOL and MG, the M-response can also be seen (\*  $p < 0.05$ , \*\*  $p < 0.01$ )

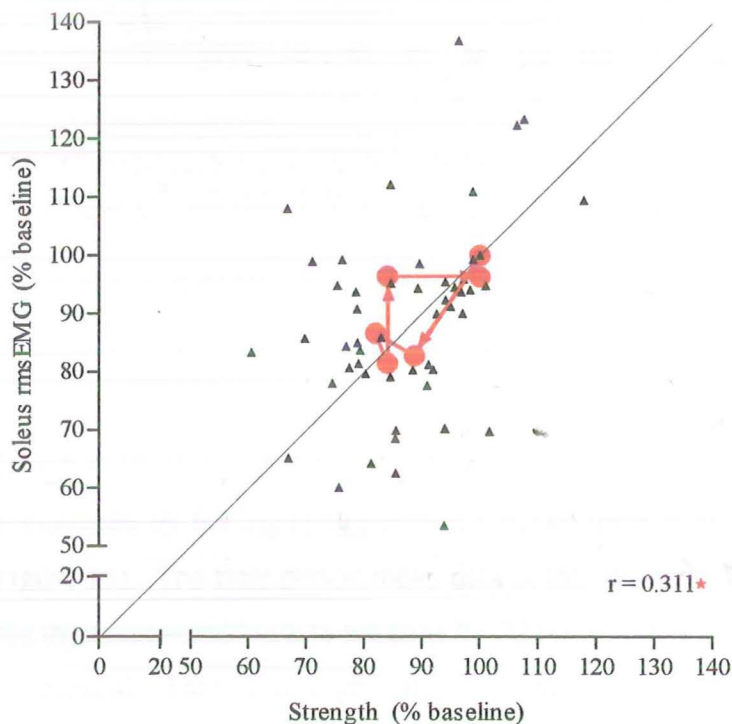
#### 4.3.2 Recovery of evoked responses

Similar to that of the MVC torque, the SOL H-reflex was still reduced one hour post-exercise at  $68.2 \pm 19.7$  of the baseline ( $p = 0.001$ ). It slowly increased over the 72 hour period but was still reduced by 7% from the pre exercise value. Although not significant, the T-wave responses post-exercise displayed a similar pattern to that of the H-reflex immediately post exercise, but increased above the baseline at 24 hours (Figure 10). The SOL H:M remained decreased one and 24 hours post exercise, but had recovered by 72 hours (Figure 11).

As seen in Figure 12, the evoked twitch showed a similar exercise effect to that of the MVC torque with the amplitude of the evoked twitch still decreased one hour post-exercise at  $86.6 \pm 16.0$  % of baseline ( $p = 0.038$ ), but showed a more rapid recovery back to baseline at 24 hours ( $96.9 \pm 28.6$ ). There were no significant changes in the HRT and TTP of the evoked twitch in the time course of recovery.

#### 4.4 Relationships between the variables

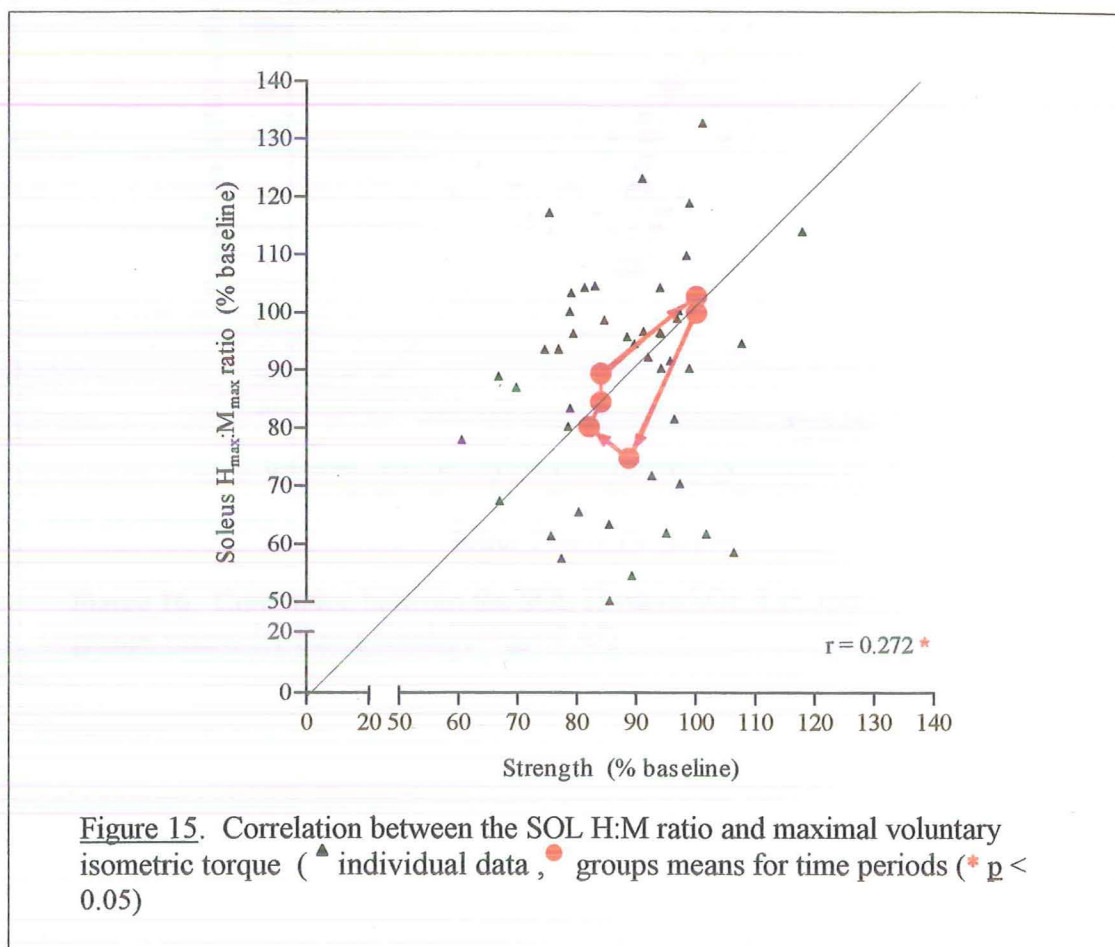
Linear correlation coefficients were calculated for all the dependent variables to determine if the evoked, voluntary, electrical, and mechanical parameters of the study were related (details in Appendix F). Significant correlations included the SOL rmsEMG and strength, SOL H-reflex and strength, SOL H:M ratio and strength, evoked twitch amplitude and strength, SOL H-reflex and SOL T-wave, and SOL H-reflex and evoked twitch amplitude. The correlation figures (Figures 14 – 16) show the individual results for all time slots and all subjects ( $\blacktriangle$ ), as well as the group mean results for each time period ( $\bullet$ ). Figure 14 shows the correlation between strength and the SOL rmsEMG with a MVC. The pattern of the mean data points over time shows a similar reduction at first, then the SOL rmsEMG recovered, declined, then recovered quickly back to baseline. In contrast, the strength remained decreased until 48 hours post exercise.



**Figure 14.** Correlation between the soleus EMG and maximal voluntary isometric torque, ( $\blacktriangle$  individual data,  $\bullet$  groups means for time periods) (\*  $p < 0.05$ )

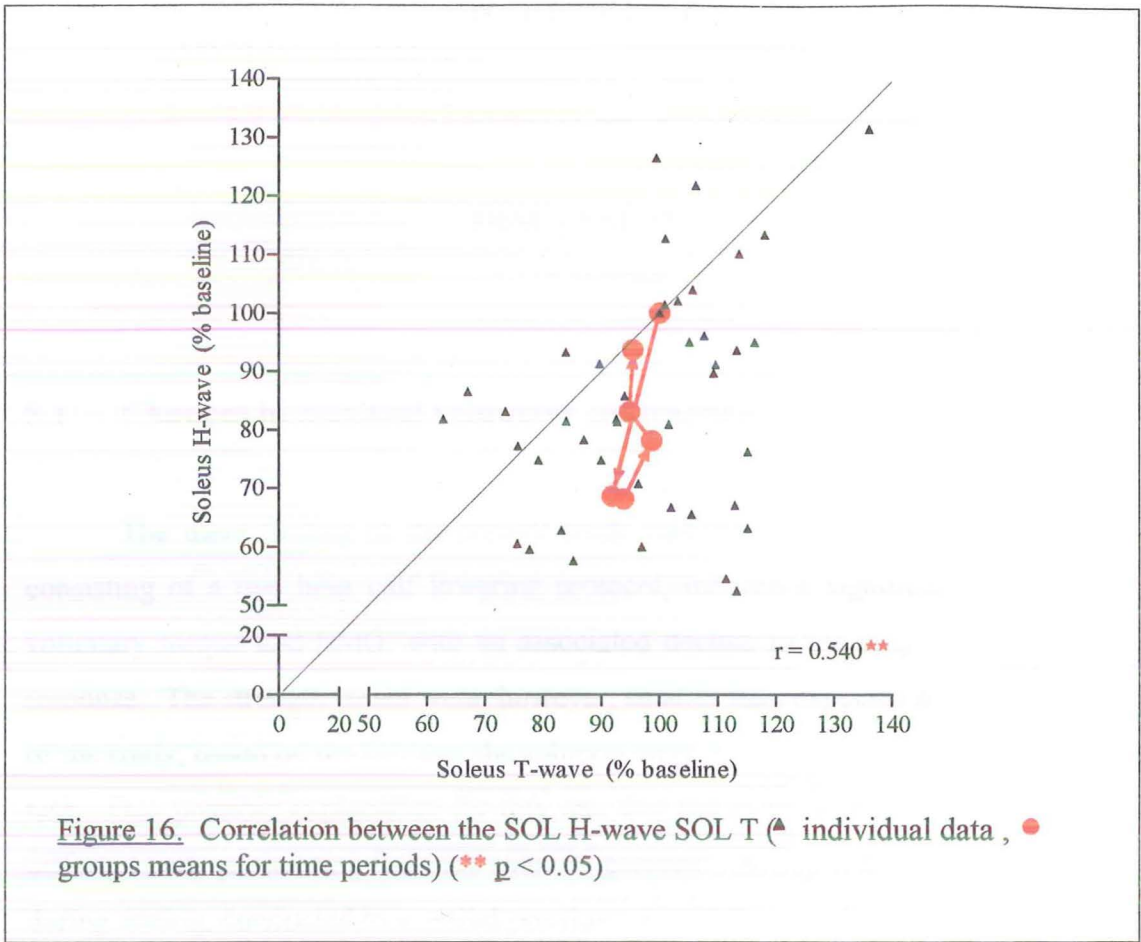


Figure 15 shows the correlation between strength and the SOL H:M ratio. A similar pattern is displayed in terms of percentage change, however the SOL H:M recovered at a slightly faster rate.



**Figure 15.** Correlation between the SOL H:M ratio and maximal voluntary isometric torque (▲ individual data, ● groups means for time periods (\*  $p < 0.05$ ))

Although there is a correlational relationship between the SOL T-wave and H-wave (as indicated by the highly significant  $r$  value) there is no similar pattern of recovery (Figure 16). The time period mean data points show the H-wave decreased post-exercise then recovered back to baseline by 72 hours, while the T-wave remains relatively unchanged. There is a large spread of data points below and above 100% of the baseline, which indicates a large variability of subject post-exercise responses.



**Figure 16.** Correlation between the SOL H-wave SOL T ( $\blacktriangle$  individual data,  $\bullet$  groups means for time periods) ( $** p < 0.05$ )

## CHAPTER FIVE

### DISCUSSION

#### 5.1 Changes in maximal voluntary contraction

The main finding of the present study was that an eccentric exercise bout, consisting of a two hour calf lowering protocol, induced a significant decrease in voluntary torque and EMG, with an associated decline in the amplitude of the H-response. The strength losses were, however, smaller than expected at the beginning of the study, based on the fact that the subjects were near exhaustion at the end of the task. One possible explanation for this was that the method of MVC testing had a different body positioning from the exercise protocol. Alway et al. (1989) found that during testing, conducted in a seated position with the knee at 90°, the gastrocnemius was in a sub-optimal position for force generation. It would have been more effective to measure maximal isometric torque on the same calf raise machine as the exercise was performed on. Although this was not possible in the present study it should be kept in mind for future studies using this model.

The observed reduction in the force generating capacity supports previous reports of strength decrement following voluntary eccentric exercise (Bentley et al., 2000; Esposito et al., 1998; Hamlin & Quigley, 2001b; Kroon & Naeije, 1991; Smith et al., 1994). Following a 20 minute stretch exercise Behm, Button, & Butt (2001) found a 12% decrease in maximal isometric strength. Bentley et al. (2000) found that following a 30 minute cycling protocol maximal voluntary force was significantly reduced post-exercise and the mechanical and electrical activities of the MU of the quadriceps were altered. In comparison with other prolonged activities in the quadriceps, the average muscular torque losses of 18% after the two hour eccentric exercise in this study were smaller than those reported after a prolonged running exercise (Sherman et al., 1984), but closer to those observed from a two hour

cycling protocol of 14% (Leppers et al., 2000). The results of this study showed that following a bout of eccentric exercise, voluntary torque fully recovered to pre-exercise values by 72 hours. Similarly, Smith et al. (1994) found the greatest reduction at 48 hours, while Hamlin & Quigley (2001b) also found force still decreased at 48 hours post exercise. This suggests that there was minimal muscle damage resulting from the exercise, which supports the relatively small CK increments measured following the eccentric exercise bout.

## **5.2 Reduction in EMG**

The decrease in MVC torque was associated with a reduction in the SOL (23%) and MG (29%) voluntary rmsEMG activity. A number of other studies have indicated that muscle fatigue leads to a decrease in the neural activation of motor units (Bigland-Ritchie, Johansson, Lippold, Smith et al., 1983; Bigland-Ritchie & Woods, 1984; Enoka & Stuart, 1992; Hakkinen, 1993; Moritani et al., 1990; Nottle & Sacco, 2002). Avela et al. (1999) found a 16.5% decrease in SOL EMG following a one hour repeated passive stretching protocol. Similarly, following a 20 minute stretch exercise Behm et al. (2001) found a 12% decrease in maximal isometric strength, a 20% decrease in EMG, and a 11% decrease in TPT, but no change in tetanic force. They concluded that the stretch-induced decrease in MVC could be partially attributed to decreases in muscle activation, and that prolonged stress on the joint receptors could possibly lead to inhibitory effects upon the MN (Behm et al., 2001). Fowles et al. (2000) found that EMG was significantly depressed after 30 minutes of cyclical passive stretching but had recovered by 15 minutes. Similarly, a reduction in integrated EMG 24 hours following a resistance exercise bout has been reported, and it was suggested the reduction in isometric force was possibly due to fatigue of central origin, as evidenced by the decreased integrated EMG level (Linnamo, Hakkinen, & Komi, 1998). The difference in the above studies with regard to the present study would likely be a result of the differing type and duration of the exercise protocols.

The EMG reductions shown in this study suggest a decreased neural drive to the muscle. The reduced neural input could imply the occurrence of central fatigue,

which can be caused either by supraspinal fatigue or by changes in the inhibitory as well as disfacilitory signals originating from the contracting muscle (Avela et al., 1999; Bigland-Ritchie, Dawson, Johansson, & Lippold, 1986). The simultaneous decrease in the firing rate and in the amplitude of the of the MU action potential and the de-recruitment of some highly fatigable MU would lead to a decrease in the EMG signal (Esposito et al., 1998).

Although decreases in MN firing rates and spinal reflexes have been demonstrated during fatigue (Bigland-Ritchie, Johansson, Lippold, & Woods, 1983; Garland & McComas, 1990), such alterations are not themselves indications of central fatigue (Gandevia et al., 1996). MN firing rates have shown to decrease during a sustained voluntary contraction but this is a functionally useful change which matches activation to the slower contractile properties of the muscle. However too great a slowing of neuronal firing would constitute central fatigue (Gandevia et al., 1996). It has been shown that because of central fatigue, maximal voluntary force will actually be less than the maximal evocable force, so that any evoked twitch force would be a larger percentage of maximal voluntary force than of the maximal evocable force (Gandevia et al., 1996). A limiting factor in the present study was that central activation ratio was not measured, therefore the voluntary force and the maximal evokable force could not be compared, however, there was no observable change in the amplitude of the twitch torque.

EMG and strength displayed differing recovery rates in the present study, with a similar reduction at first, but no similarities thereafter. Kroon & Naeije (1991) found the recovery rate of the EMG was similar to the rate of recovery of muscle performance up to 25hours after dynamic exercise of the human biceps muscle to exhaustion, with the MVC force decreased until four days post-exercise and EMG altered for 10 days post an eccentric exercise bout until exhaustion. Kukulka, Russell, & Moore (1986) found that the changes in electrical activation and force generating capabilities of soleus during sustained, maximum isometric efforts were all consistent with a muscle designed to optimally resist fatigue. It has been argued that the slowing of MN firing in response to fatigue may act as a compensatory mechanism for preserving optimum force output and limiting NM block. A reduction on neural firing proportional to the prolongation of the muscle twitch, would aid in force being maintained at maximum tetanic levels without

unwarranted neural drive (Kukulka, Russell, & Moore, 1986; McHugh, Connolly, Eston, & Gleim, 2000).

### **5.3 Neuromuscular propagation and Excitation-Contraction coupling**

A possible explanation for the reduction in force following the eccentric exercise protocol could be either a failure in, or weakened, NM propagation. However, the possibility of weakened NM propagation is excluded by the nonsignificant changes in the maximal M-wave in the present study. Nottle & Sacco (2002) found no alteration in the SOL or MG M-wave following a bout of repeated submaximal eccentric contractions of downhill walking. Bigland-Ritchie, Johansson, Lippold, & Woods (1983) also found that during a 60 second MVC there was a decrease in the EMG but not in the M-wave, and concluded that the reduction in force was not due to a NM block, but due to a reduction in the firing pattern of the MN pool. It appears that the MN firing rates elicited by voluntary effort is regulated and limited for each muscle to the minimum requirement for maximal force generation, therefore preventing NM transmission failure and optimising motor control (Bigland-Ritchie & Woods, 1984). Nevertheless, the alteration of M-wave in human muscle with fatigue is still controversial. An increase in M-wave following a bout of 70 repeated eccentric contractions has been reported (Hortobagyi, Tracy, Hamilton, & Lambert, 1996), while a decreased M-wave post-exercise has also been shown with sustained submaximal contractions (Fuglevand et al., 1993), a prolonged cycling exercise (Lepers et al., 2000), and an acute eccentric resistance bout (Michaut et al., 2002). This is largely due to the differences in tasks and type of contractions performed to induce fatigue.

Another possible explanation for the reduction in force due to the exercise protocol is an alteration in the excitation-contraction coupling (E-C coupling) process, and is demonstrated by a large number of studies of muscle damage induced by eccentric exercise (Davies & White, 1981; Esposito et al., 1998; Hamlin & Quigley, 2001a; Newham, Mills et al., 1983; Pearce et al., 1998). It has been argued that eccentric contractions damage the contractile machinery causing a force deficit, and are not associated with a reduction in excitation as assessed by surface EMG.

The reduction in twitch torque found in the present study tends to support the notion of an altered E-C coupling. However, since the twitch torque had recovered by 24 hours, while voluntary strength and EMG remained reduced, some portion of the strength loss may be related to E-C coupling failure, but can be attributed to other mechanisms also. Further studies will require quantifying E-C coupling change by following alterations in twitch torque ratios during recovery from such exercise.

#### **5.4 Decrease in $\alpha$ -motorneuron excitability**

Similar to that of the MVC torque, the SOL H-reflex and H:M ratio were still reduced by 32% and 25% respectively at one hour post-exercise and slowly recovered back to baseline over 72 hours. The significant decrease in the SOL H:M following the eccentric exercise protocol supports previous research, however the extent of the reduction of the H:M ratio in the present study is smaller than previous reports (Avela et al., 1999; Garland & McComas, 1990; Trimble & Harp, 1998). Trimble & Harp (1998) found a 36% decrease in the SOL  $H_{max}:M_{max}$ , as well as a potentiation of the lateral gastrocnemius H:M for 10 minutes post a concentric-eccentric exercise bout, while Garland & McComas (1990) found a 47% reduction following electrically induced fatigue. Similarly, Avela et al. (1999) found a 44% decrease following a one hour repeated passive stretching condition and concluded that the reduction was due to a decline in stretch reflex sensitivity and the decreased  $\alpha$ -motorneuron pool excitability. Only a 12% reduction in the H:M was found by Bulbulian & Darabos (1986) following low intensity exercise. They concluded that the highly significant change was due to a tranquillising response under conditions of high intensity exercise.

The H-reflex reflects the amplitude of the net excitability and inhibitory influences in the  $\alpha$ -motorneuron pool (Leonard et al., 1994). However, as modulation of reflex amplitude is somewhat independent of central drive this indicates that reflex magnitude is not merely a reflection of motorneuron excitability, but can also be influenced by additional neural mechanisms (Pinniger et al., 2001). In general, the size of the H-reflex is affected by the ongoing net excitatory drive onto the  $\alpha$ -motorneurons, a reduced H-reflex represents either a reduced excitatory

drive to the  $\alpha$ -motoneurons, or an enhanced inhibitory effect (Avela et al., 1999). Alpha MNs receive monosynaptic and polysynaptic input from sensorimotor cortical projections, brain stem nuclei, and type Ia, Ib, II, III and IV sensory afferents (Enoka, 1994), and can be seen with Figure 17, a model of the inputs and outputs to the  $\alpha$ -motoneurons.

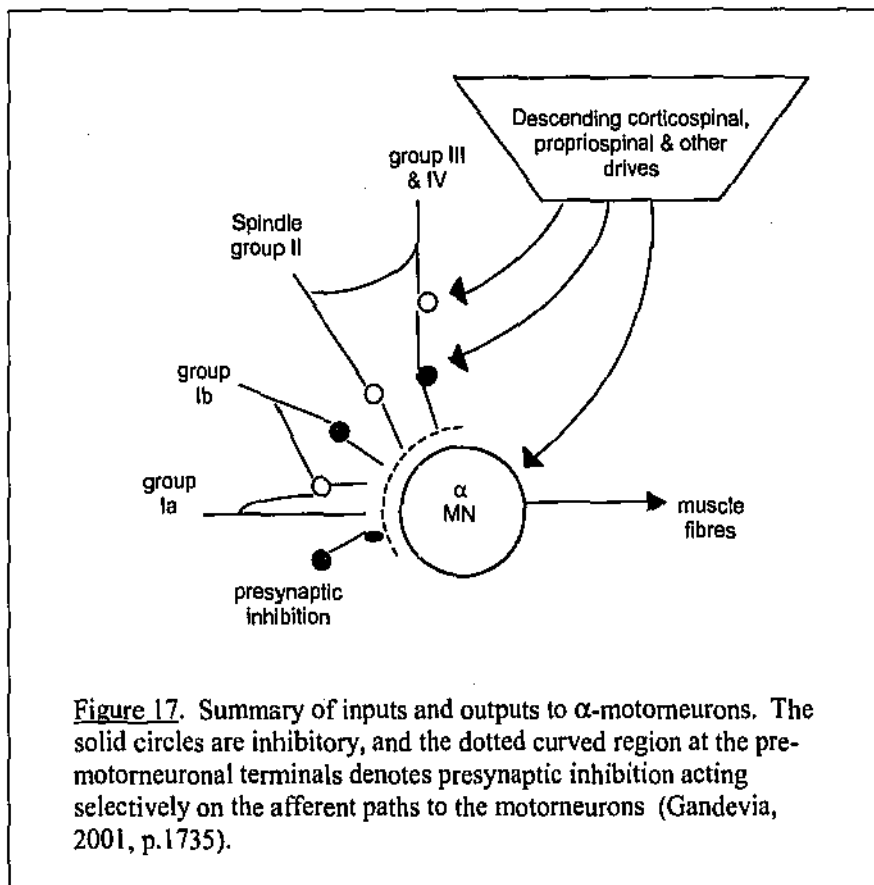
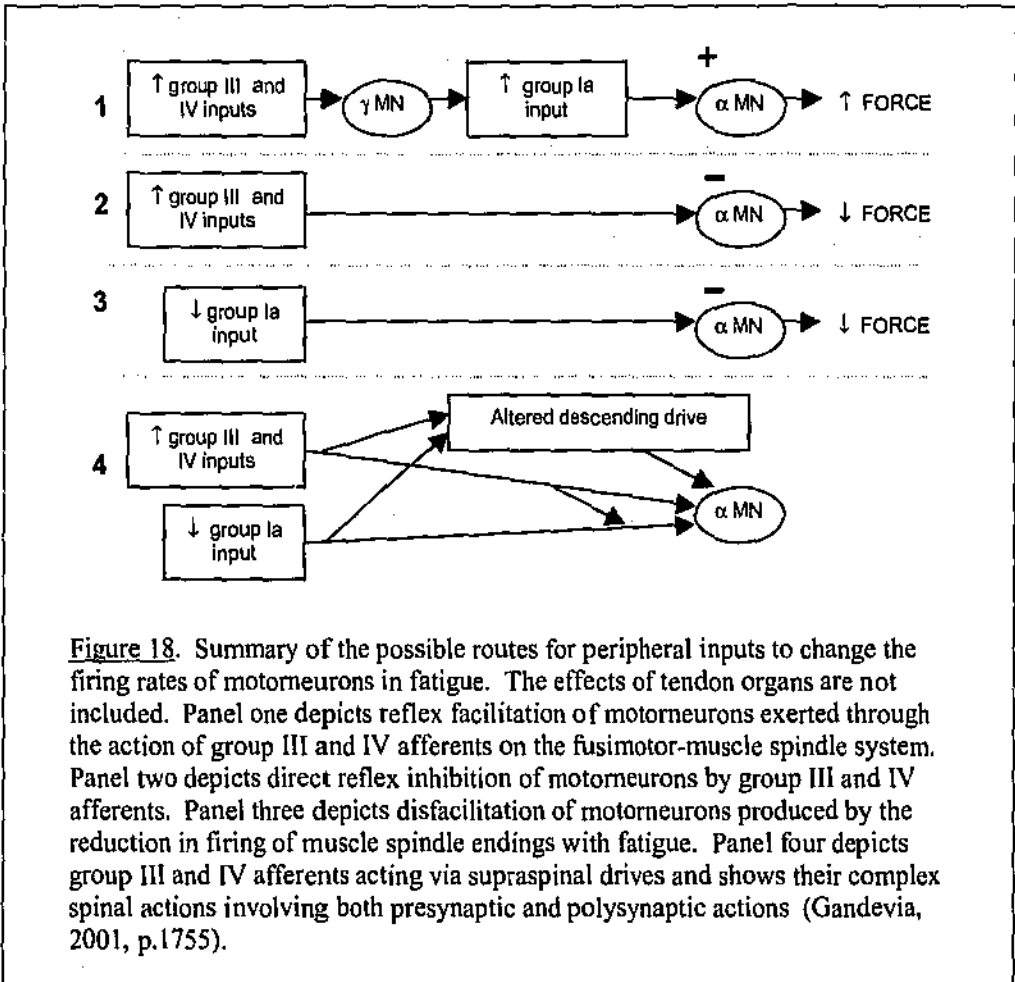


Figure 18 represents the possible routes for peripheral input to change motoneurons (and force production) with fatigue. Panel one shows the activation of III, IV and Ia afferents reinforcing muscle contraction through activation of the fusimotor ( $\gamma$  MN) path, Panel two summarises the view of H-reflex testing after fatigue, Panel three depicts the disfacilitation accompanying a decline in spindle input during sustained isometric contractions, and Panel four shows a more complex explanation of force modulation based on the presynaptic, spinal and supraspinal action of group III and IV afferents (Gandevia, 2001).





There is no *a priori* reason why the declines in EMG and reflex excitability should be identical. There are probably many important differences in the spinal circuitry involved in the H-reflex and the descending drive onto motoneurons, and in the postsynaptic responses of the motoneurons to the two forms of excitatory command. Such differences could cause a fatigue-induced reduction in motoneuronal excitability to affect the EMG and H-reflex excitability to unequal extents, particularly when the potential effects of presynaptic inhibitory circuits are taken into account (Garland & McComas, 1990).

Therefore a number of mechanisms could account for the prolonged post-exercise decrease in the H-reflex seen in this study. One possibility is presynaptic inhibition. Pre-synaptic factors affecting the  $\alpha$ -motoneurons are the extrinsic

properties of a MN and include factors such as the number of synaptic terminals per MN from a given input system, the spatial distribution of synaptic terminals (Funase et al., 1994). Inhibition of the H-reflex may be attributed to several mechanisms. These may include the inability to evoke volleys in Ia fibres, and a reduced probability of transmitter release from the presynaptic terminal (homosynaptic post-activation depression). Presynaptic inhibition of Ia afferents from plantar flexor agonists, the origin of which is possibly the decreased resting discharge of the muscle spindles because of increased compliance of the muscle from the eccentric contractions, would lead to a reduced H-reflex (Pinniger et al., 2001). Also, group Ib, and spindle group II along with special cutaneous afferents receive abundant presynaptic contacts capable of mediating presynaptic inhibition (Gandevia, 2001). The influence of Ib afferents could be considered to be the likely cause of reflex modulation as Ib afferents are sensitive to very small changes in muscle tension and are influential during active muscle (Pinniger et al., 2001). Group II afferents are predominantly regarded as indicators of static length changes; therefore, the influence of muscle spindle discharge on reflex modulation has been found to arise from predominantly Ia afferents (Pinniger et al., 2001), and group III afferents innervating tendons are plentiful and may exert presynaptic inhibition on the group Ia fibres (Priori et al., 1998). The presynaptic inhibition of the Ia afferent terminals due to stimulation of the group III and IV muscle afferents may be a valid explanation for the H-reflex depression, although some other forms of inhibition could also be involved (Avela et al., 1999). The intrinsic properties of the motoneuron may change with fatiguing exercise, but the examination of this phenomenon goes beyond the scope of this study.

Another possible explanation for the decreased H-reflex with fatigue is due to postsynaptic mechanisms. The post-synaptic factors are the intrinsic properties of a MN and include the total membrane area, electronic architecture of the MN which depends on the cell anatomy, the membrane time constant (Funase et al., 1994). Finally, a third possible explanation is that tonic pain can influence the motor system. It has been found that decreases in the H-reflex 20 minutes after the disappearance of pain was due to a reduction in the excitability of the cortical and spinal motoneurons (Le Pera et al., 2001), although this is unlikely due to the nature of the exercise bout

in the present study. Whatever factors are responsible for reducing excitability in this model must be long lasting, as recovery is complete only after 72 hours.

To conclude, the findings suggest the decrease in  $\alpha$ -motoneuron excitability due to presynaptic inhibition from the III and IV afferents, a decrease in Ia afferent output and inhibition from the exercising muscle. Voluntary strength, EMG and the reflex excitability of the  $\alpha$ -motoneuron pool were all significantly depressed following fatigue of the plantar flexors induced by an eccentric exercise bout, and the respective depressions could not be explained by peripheral failure, or reduced neural drive alone. With fatigue there is likely to be a net reduction in spinal reflex facilitation and increase in inhibition, thus the motoneurons are harder to drive by volition.

## 5.5 Tendon reflex

The increase in spindle excitability following an exercise protocol is usually reflected by an increase in the tendon reflex. The amplitude and rate of stretch of the muscle depend both on the mechanical features of the stimulus (site of impact, angle of impact, force delivered) and on the compliance of the muscle tissue (Brunia, 1973). In the present study there was a 30% reduction in H-wave, therefore a similar decrease would be expected in the T-wave as the action potentials travel along the Ia afferents to the spinal cord and induce a reflex response (T-wave) of  $\alpha$ -motoneurons leading to a twitch of the muscle. Unexpectedly, the T-reflex remained virtually unchanged. It is possible that the tendon response could have increased as a result of increased muscle compliance, but showed no change due to an increase in inhibition (as shown by a decreased H-reflex). Another possible explanation, is that the small-diameter afferents (rather than the activity of the large-diameter axons) resulting from the reduced sensitivity of the muscle spindles to stretch, lead to a modulation of the T-wave (Avela et al., 1999). The findings lead to the suggestion that with further research a number of variables should be tested when using the Tendon tap as a measure of  $\alpha$ -motoneuron excitability.

## **5.6 Relationship between the variables**

Of the variables examined significant correlations were found for strength and the SOL H-reflex, H:M, T-reflex, and EMG, SOL H:M and EMG, and SOL H-reflex and SOL T-reflex. This suggests that the above variables are associated with each other and/or modulated similarly. However, the relative weakness of the relationships suggest that a combination of factors also make a large contribution to strength changes induced by fatigue, and can be explained by the mechanisms described earlier.

## **5.7 Conclusions**

A bout of eccentric exercise of the triceps surae resulted in a strength loss of 18% (which recovered by 72 hours), and a reduction in the H-reflex of 30%, which remained declined at 72 hours. The respective depressions could not be explained by peripheral failure, or reduced motor activation alone. The decrease in torque and EMG with an MVC suggest force loss due to a decreased neural drive, there was a change in twitch peak torque, but it recovered by 24 hours. The decline in voluntary EMG activity could not be explained by loss of excitability of NMJs or muscle fibre membranes. Although the small decline in the maximal M-wave indicated the presence of altered muscle fibre membrane or of slowed impulse conduction, this was much less than the fall in voluntary EMG, and the decrease in the H-reflex indicates a decrease in excitability of the  $\alpha$ -motoneuron pool.

The most likely explanation for the prolonged depression of the H-reflex is a reduction in the excitatory drive from the Ia afferents, and elevated presynaptic inhibition to the  $\alpha$ -motoneurons. Therefore, the decrease in MVC force was likely due to a decreased spinal excitability as a result of fatigue. The lack of agreement for changes in the T-reflex and H-reflex during recovery may be due to a decrease in spinal excitability, but an increase in spindle sensitivity and compliance brought about by the nature of the contractions, lead to a net change of zero. Result suggests that alterations in motor drive associated with fatiguing eccentric exercise probably

represent a combination of the modulatory effects of a number of inputs (both excitatory and inhibitory) to the  $\alpha$ -motoneuron.

The exercise protocol used in this study was unique, with the results suggesting that the prolonged eccentric exercise bout was sufficient to impair the central and peripheral mechanisms of force generation in plantarflexors for a period of 72 hours. This has implications for athletes when planning their exercise programs, as the mechanisms of fatigue and recovery in specific training regimes should be identified for an optimal training program, particularly when planning training sessions around competitions. It would be interesting to further investigate this concept, incorporating the limitations of the present study, by looking at the effect of muscle function and motoneuron excitability with eccentric exercise bouts of different intensity levels.

## REFERENCES

- Alway, S. E., MacDougall, J. D., & Sale, D. G. (1989). Contractile adaptations in the human triceps surae after isometric exercise. Journal of Applied Physiology, *66*(6), 2725-2732.
- Avela, J., Kyrolainen, H., & Komi, P. V. (1999). Altered reflex sensitivity after repeated and prolonged passive muscle stretching. Journal of Applied Physiology, *86*(4), 1283-1291.
- Behm, D. G., Button, D. C., & Butt, J. C. (2001). Factors affecting force loss with prolonged stretching. Canadian Journal of Applied Physiology, *26*(3), 261-272.
- Bentley, D. J., Smith, P. A., Davie, A. J., & Zhou, S. (2000). Muscle activation of the knee extensors following high intensity endurance exercise in cyclists. European Journal of Applied Physiology, *81*(4), 297-302.
- Bigland-Ritchie, B. (1981a). EMG and fatigue of human voluntary and stimulated contractions, Human muscle fatigue: physiological mechanisms (Vol. 82, pp. 130-156). London (Ciba Foundation Symposium): Pitman Medical.
- Bigland-Ritchie, B. (1981b). EMG/force relations and fatigue of human voluntary contractions. Exercise & Sport Sciences Reviews, *9*, 75-117.
- Bigland-Ritchie, B., Dawson, N. J., Johansson, R. S., & Lippold, O. C. (1986). Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. Journal of Physiology, *379*, 451-459.
- Bigland-Ritchie, B., Johansson, R., Lippold, O., Smith, S., & Woods, J. J. (1983). Changes in motoneuron firing rates during sustained maximal voluntary contractions. Journal of Physiology, *340*, 335-346.
- Bigland-Ritchie, B., Johansson, R., Lippold, O. C., & Woods, J. J. (1983). Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. Journal of Neurophysiology, *50*(1), 313-324.
- Bigland-Ritchie, B., Jones, D. A., & Woods, J. J. (1979). Excitation frequency and muscle fatigue: electrical responses during human voluntary and stimulated contractions. Experimental Neurology, *64*(2), 414-427.
- Bigland-Ritchie, B., & Woods, J. J. (1984). Changes in muscle contractile properties and neural control during human muscular fatigue. Muscle & Nerve, *7*(9), 691-699.

- Binder-Macleod, S. A., & Snyder-Mackler, L. (1993). Muscle fatigue: clinical implications for fatigue assessment and neuromuscular electrical stimulation. Physical Therapy, 73(12), 902-910.
- Brunia, C. H. M. (1973). A Discussion of the Methodology of the Triceps Surae T- and H-Reflexes (Vol. 3). Basel, New York: S. Karger.
- Bulbulian, R., & Darabos, B. L. (1986). Motor neuron excitability: the Hoffmann reflex following exercise of low and high intensity. Medicine & Science in Sports & Exercise, 18(6), 697-702.
- Butler, A. J., Yue, G., & Darling, W. G. (1993). Variations in soleus H-reflexes as a function of plantarflexion torque in man. Brain Research, 632(1-2), 95-104.
- Clarkson, P. M., Byrnes, W. C., McCormick, K. M., Turcotte, L. P., & White, J. S. (1986). Muscle soreness and serum creatine kinase activity following isometric, eccentric, and concentric exercise. International Journal of Sports Medicine, 7(3), 152-155.
- Clarkson, P. M., Kroll, W., & McBride, T. C. (1980). Plantar flexion fatigue and muscle fiber type in power and endurance athletes. Medicine & Science in Sports & Exercise, 12(4), 262-267.
- Crenna, P., & Frigo, C. (1987). Excitability of the soleus H-reflex arc during walking and stepping in man. Experimental Brain Research, 66(1), 49-60.
- Crone, C., Hultborn, H., Mazieres, L., Morin, C., Nielsen, J., & Pierrot-Deseilligny, E. (1990). Sensitivity of monosynaptic test reflexes to facilitation and inhibition as a function of the test reflex size: a study in man and the cat. Experimental Brain Research, 81(1), 35-45.
- Davies, C. T., & White, M. J. (1981). Muscle weakness following eccentric work in man. Pflugers Archiv - European Journal of Physiology, 392(2), 168-171.
- Davis, J. M. (1995). Central and peripheral factors in fatigue. Journal of Sports Sciences, 13, S49-53.
- Dolezal, B. A., Potteiger, J. A., Jacobsen, D. J., & Benedict, S. H. (2000). Muscle damage and resting metabolic rate after acute resistance exercise with an eccentric overload. Medicine & Science in Sports & Exercise, 32(7), 1202-1207.
- Ellrich, J., Steffens, H., Treede, R. D., & Schomburg, E. D. (1998). The Hoffmann reflex of human plantar foot muscles. Muscle & Nerve, 21(6), 732-738.
- Enoka, R. M. (1994). Neuromechanical Basis of Kinesiology (2nd Ed.). Champaign, IL: Human Kinetics.

- Enoka, R. M., Hutton, R. S., & Eldred, E. (1980). Changes in excitability of tendon tap and Hoffmann reflexes following voluntary contractions. Electroencephalography & Clinical Neurophysiology, 48(6), 664-672.
- Enoka, R. M., & Stuart, D. G. (1992). Neurobiology of muscle fatigue. Journal of Applied Physiology, 72(5), 1631-1648.
- Esposito, F., Orizio, C., & Veicsteinas, A. (1998). Electromyogram and mechanomyogram changes in fresh and fatigued muscle during sustained contraction in men. European Journal of Applied Physiology & Occupational Physiology, 78(6), 494-501.
- Fowles, J. R., Sale, D. G., & MacDougall, J. D. (2000). Reduced strength after passive stretch of the human plantarflexors. Journal of Applied Physiology, 89(3), 1179-1188.
- Fuglevand, A. J., Zackowski, K. M., Huey, K. A., & Enoka, R. M. (1993). Impairment of neuromuscular propagation during human fatiguing contractions at submaximal forces. Journal of Physiology, 460, 549-572.
- Funase, K., Imanaka, K., & Nishihira, Y. (1994). Excitability of the soleus motoneuron pool revealed by the developmental slope of the H-reflex as reflex gain. Electromyography & Clinical Neurophysiology, 34(8), 477-489.
- Gandevia, S. C. (2001). Spinal and supraspinal factors in human muscle fatigue. Physiological Reviews, 81(4), 1725-1789.
- Gandevia, S. C., Allen, G. M., Butler, J. E., & Taylor, J. L. (1996). Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. Journal of Physiology, 490(Pt 2), 529-536.
- Garland, S. J., & McComas, A. J. (1990). Reflex inhibition of human soleus muscle during fatigue. Journal of Physiology, 429, 17-27.
- Garrett, M., & Caulfield, B. (2001). Increased H(max):M(max) ratio in community walkers poststroke without increase in ankle plantarflexion during walking. Archives of Physical Medicine & Rehabilitation, 82(8), 1066-1072.
- Gibala, M. J., MacDougall, J. D., Tarnopolsky, M. A., Stauber, W. T., & Elorriaga, A. (1995). Changes in human skeletal muscle ultrastructure and force production after acute resistance exercise. Journal of Applied Physiology, 78(2), 702-708.
- Gravel, D., Belanger, A. Y., & Richards, C. L. (1987). Study of human muscle contraction using electrically evoked twitch responses during passive shortening and lengthening movements. European Journal of Applied Physiology & Occupational Physiology, 56(6), 623-627.



- Grimby, L., Hannerz, J., Borg, J., & Hedman, B. (1981). Firing properties of single human motor units on maintained maximal voluntary effort, Human muscle fatigue: physiological mechanisms (Vol. 82, pp. 157-177). London (Ciba Foundation Symposium): Pitman Medical.
- Hakkinen, K. (1993). Neuromuscular fatigue and recovery in male and female athletes during heavy resistance exercise. International Journal of Sports Medicine, 14(2), 53-59.
- Hakkinen, K. (1995). Neuromuscular fatigue and recovery in women at different ages during heavy resistance loading. Electromyography & Clinical Neurophysiology, 35(7), 403-413.
- Hamlin, M. J., & Quigley, B. M. (2001a). Quadriceps concentric and eccentric exercise 1: changes in contractile and electrical activity following eccentric and concentric exercise. Journal of Science & Medicine in Sport, 4(1), 88-103.
- Hamlin, M. J., & Quigley, B. M. (2001b). Quadriceps concentric and eccentric exercise 2: differences in muscle strength, fatigue and EMG activity in eccentrically-exercised sore and non-sore muscles. Journal of Science & Medicine in Sport, 4(1), 104-115.
- Hoffman, M. A., & Koceja, D. M. (1995). The effects of vision and task complexity on Hoffmann reflex gain. Brain Research, 700(1-2), 303-307.
- Hortobagyi, T., Tracy, J., Hamilton, G., & Lambert, J. (1996). Fatigue effects on muscle excitability. International Journal of Sports Medicine, 17(6), 409-414.
- Ingalls, C. P., Warren, G. L., Williams, J. H., Ward, C. W., & Armstrong, R. B. (1998). E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. Journal of Applied Physiology, 85(1), 58-67.
- Jones, D. A. (1981). Muscle fatigue due to changes beyond the neuromuscular junction, Human muscle fatigue: physiological mechanisms (Vol. 82, pp. 178-196). London (Ciba Foundation Symposium): Pitman Medical.
- Kent-Braun, J. A., & Le Blanc, R. (1996). Quantitation of central activation failure during maximal voluntary contractions in humans. Muscle & Nerve, 19(7), 861-869.
- Kirsch, R. F., & Rymer, W. Z. (1987). Neural compensation for muscular fatigue: evidence for significant force regulation in man. Journal of Neurophysiology, 57(6), 1893-1910.

- Kroon, G. W., & Naeije, M. (1991). Recovery of the human biceps electromyogram after heavy eccentric, concentric or isometric exercise. European Journal of Applied Physiology & Occupational Physiology, 63(6), 444-448.
- Kukulka, C. G., Russell, A. G., & Moore, M. A. (1986). Electrical and mechanical changes in human soleus muscle during sustained maximum isometric contractions. Brain Research, 362(1), 47-54.
- Latash, M. L. (1998). Neurophysiological basis of movement: Champaign, IL: Human Kinetics.
- Le Pera, D., Graven-Nielsen, T., Valeriani, M., Oliviero, A., Di Lazzaro, V., Tonali, P. A., & Arendt-Nielsen, L. (2001). Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain. Clinical Neurophysiology, 112(9), 1633-1641.
- Leonard, C. T., Kane, J., Perdaems, J., Frank, C., Graetzer, D. G., & Moritani, T. (1994). Neural modulation of muscle contractile properties during fatigue: afferent feedback dependence. Electroencephalography & Clinical Neurophysiology, 93(3), 209-217.
- Lepers, R., Hausswirth, C., Maffiuletti, N., Brisswalter, J., & van Hoecke, J. (2000). Evidence of neuromuscular fatigue after prolonged cycling exercise. Medicine & Science in Sports & Exercise, 32(11), 1880-1886.
- Linnamo, V., Hakkinen, K., & Komi, P. V. (1998). Neuromuscular fatigue and recovery in maximal compared to explosive strength loading. European Journal of Applied Physiology & Occupational Physiology, 77(1-2), 176-181.
- Maffiuletti, N. A., Martin, A., Babault, N., Pensini, M., Lucas, B., & Schieppati, M. (2001). Electrical and mechanical H(max)-to-M(max) ratio in power- and endurance-trained athletes. Journal of Applied Physiology, 90(1), 3-9.
- McHugh, M. P., Connolly, D. A., Eston, R. G., Gattman, E. J., & Gleim, G. W. (2001). Electromyographic analysis of repeated bouts of eccentric exercise. Journal of Sports Sciences, 19(3), 163-170.
- McHugh, M. P., Connolly, D. A., Eston, R. G., & Gleim, G. W. (2000). Electromyographic analysis of exercise resulting in symptoms of muscle damage. Journal of Sports Sciences, 18(3), 163-172.
- Michaut, A., Pousson, M., Babault, N., & Van Hoecke, J. (2002). Is eccentric exercise-induced torque decrease contraction type dependent? Medicine & Science in Sports & Exercise, 34(6), 1003-1008.

- Moritani, T., Oddson, L., & Thorstensson, A. (1990). Electromyographic evidence of selective fatigue during the eccentric phase of stretch/shortening cycles in man. European Journal of Applied Physiology & Occupational Physiology, 60(6), 425-429.
- Newham, D. J., Jones, D. A., & Clarkson, P. M. (1987). Repeated high-force eccentric exercise: effects on muscle pain and damage. Journal of Applied Physiology, 63(4), 1381-1386.
- Newham, D. J., Jones, D. A., & Edwards, R. H. (1983). Large delayed plasma creatine kinase changes after stepping exercise. Muscle & Nerve, 6(5), 380-385.
- Newham, D. J., Mills, K. R., Quigley, B. M., & Edwards, R. H. (1983). Pain and fatigue after concentric and eccentric muscle contractions. Clinical Science, 64(1), 55-62.
- Norman, G. R., & Streiner, D. L. (1999). PDQ Statistics (2nd ed.). Hamilton, Ontario: B.C. Decker Inc.
- Nottle, C., & Sacco, P. (2002). Electromyographic (EMG) and torque changes in triceps surae following repeated submaximal eccentric contractions. Journal of Science & Medicine in Sport, 5(4), 110.
- Pearce, A. J., Sacco, P., Byrnes, M. L., Thickbroom, G. W., & Mastaglia, F. L. (1998). The effects of eccentric exercise on neuromuscular function of the biceps brachii. Journal of Science & Medicine in Sport, 1(4), 236-244.
- Pinniger, G. J., Nordlund, M., Steele, J. R., & Cresswell, A. G. (2001). H-reflex modulation during passive lengthening and shortening of the human triceps surae. Journal of Physiology, 534(3), 913-923.
- Priori, A., Berardelli, A., Inghilleri, M., Pedace, F., Giovannelli, M., & Manfredi, M. (1998). Electrical stimulation over muscle tendons in humans. Evidence favouring presynaptic inhibition of Ia fibres due to the activation of group III tendon afferents. Brain, 121(Pt 2), 373-380.
- Raastad, T., & Hallen, J. (2000). Recovery of skeletal muscle contractility after high- and moderate-intensity strength exercise. European Journal of Applied Physiology, 82(3), 206-214.
- Ross, A., Leveritt, M., & Riek, S. (2001). Neural influences on sprint running: training adaptations and acute responses. Sports Medicine, 31(6), 409-425.
- Sacco, P., Thickbroom, G. W., Byrnes, M. L., & Mastaglia, F. L. (2000). Changes in corticomotor excitability after fatiguing muscle contractions. Muscle & Nerve, 23(12), 1840-1846.

- Saxton, J. M., Clarkson, P. M., James, R., Miles, M., Westerfer, M., Clark, S., & Donnelly, A. E. (1995). Neuromuscular dysfunction following eccentric exercise. Medicine & Science in Sports & Exercise, 27(8), 1185-1193.
- Sherman, W. M., Armstrong, L. E., Murray, T. M., Hagerman, F. C., Costill, D. L., Staron, R. C., & Ivy, J. L. (1984). Effect of a 42.2-km footrace and subsequent rest or exercise on muscular strength and work capacity. Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology, 57(6), 1668-1673.
- Smith, L. L., Fulmer, M. G., Holbert, D., McCammon, M. R., Houmard, J. A., Frazer, D. D., Nsien, E., & Israel, R. G. (1994). The impact of a repeated bout of eccentric exercise on muscular strength, muscle soreness and creatine kinase. British Journal of Sports Medicine, 28(4), 267-271.
- Stackhouse, S. K., Stevens, J. E., Lee, S. C., Pearce, K. M., Snyder-Mackler, L., & Binder-Macleod, S. A. (2001). Maximum voluntary activation in nonfatigued and fatigued muscle of young and elderly individuals. Physical Therapy, 81(5), 1102-1109.
- Stephens, J. A., & Taylor, A. (1972). Fatigue of maintained voluntary muscle contraction in man. Journal of Physiology, 220(1), 1-18.
- Trimble, M. H., & Harp, S. S. (1998). Postexercise potentiation of the H-reflex in humans. Medicine and science in sports and exercise, 30(6), 933-941.

**APPENDIX A:**

**INFORMED CONSENT FORM**



**INFORMED CONSENT FORM**

**"Changes to the neural drive and motor neuron excitability following eccentric exercise causing muscle fatigue of the Triceps Surae"**

Thank you for agreeing to be a participant in my research into the area of muscle fatigue. The aim of presenting you with the following information is to inform you of the nature of the study and the tasks you will be completing during the testing period. The research aim is to determine whether muscle excitability decreases following a bout of eccentric exercise that causes muscle fatigue in the lower leg. I am interested in the relationship between central muscle fatigue, motoneuron excitability, stretch reflex and voluntary muscle contraction following exercise.

As a subject you will be asked to complete an exercise task that will involve fatiguing the muscle of the lower leg by performing a three sets of calf raises on a specifically designed calf raise machine. Muscle soreness may be experienced in the days following each exercise task.

There are four tests that you will be asked to complete on the testing days (prior to, immediately after, and three days following each of the two exercise tasks).

1. A Maximal Voluntary Contraction (MVC) test to determine your maximal calf strength.
2. A tendon tap test of the Achilles tendon to determine your calf stretch reflex.
3. Testing for motor unit excitability by electrical stimulation (some discomfort may be experienced, but it is of very short duration).
4. And testing for Creatine Kinase via a small blood sample to measure the amount of muscle damage.

You will be familiarised with the testing procedures before you begin testing so that you are fully aware of the procedures involved. All personal information and test results will remain confidential and will not be used for any purpose other than the current study.

As the study involves an exercise task and assesses changes over time, it is asked that you do not make major changes to your diet and that you don't participate in exercise during the testing period. Due to the nature of the study, it is required that subjects are healthy at the time of testing, therefore it is asked that you complete a medical and physical activity questionnaire prior to the commencement of testing.

Participation in this study is voluntary and you may withdraw at any time, for any reason. If there are any questions relating to the above information please feel free to contact me for clarification or information.

Sincerely,

Mikala Pougnault  
 Postgraduate student  
 School of Biomedical and Sport Science  
 Edith Cowan University  
 Phone: [REDACTED]  
 E-mail: [m.pougnault@ecu.edu.au](mailto:m.pougnault@ecu.edu.au)

Dr Paul Sacco  
 Supervisor  
 School of Biomedical and Sport Science  
 Edith Cowan University  
 Phone: 9400 5539  
 E-mail: [p.sacco@ecu.edu.au](mailto:p.sacco@ecu.edu.au)

I \_\_\_\_\_ have read the informed content, have completed a medical and physical activity questionnaire and have had all questions relating to the study answered.

I agree to participate in this study realising that I can withdraw at any time without prejudice. I agree that the research data obtained from this study may be published, provided I am not identifiable in any way.

Participant: \_\_\_\_\_ Date: \_\_\_\_ / \_\_\_\_ / \_\_\_\_

Investigator: \_\_\_\_\_ Date: \_\_\_\_ / \_\_\_\_ / \_\_\_\_

**APPENDIX B:**

**MEDICAL QUESTIONNAIRE**

## MEDICAL QUESTIONNAIRE

The following questionnaire is designed to establish a background of your medical history, and identify any health factor that may influence your performance.  
All information is strictly confidential.

### Personal Details

Name: \_\_\_\_\_  
Gender: M / F

Date of Birth: \_\_\_\_\_

### Medical History

Have you had / do you have any of the following?      If YES, please list the details

High or abnormal blood pressure	Y / N	_____
High cholesterol	Y / N	_____
Rheumatic fever	Y / N	_____
Heart abnormalities	Y / N	_____
Asthma	Y / N	_____
Diabetes	Y / N	_____
Epilepsy	Y / N	_____
Back / neck pain	Y / N	_____
Severe allergies	Y / N	_____
Dizziness / Fainting	Y / N	_____
Infectious diseases	Y / N	_____
Neurological disorders	Y / N	_____
Neuromuscular disorders	Y / N	_____
Are you on any medications?	Y / N	_____
Have you been injured recently?	Y / N	_____
Have you done any exercise training in the last six months?	Y / N	_____
Is there any other condition not mentioned which may affect your performance?	Y / N	_____

### Family History

Do any of the following exist in your family?      If YES, please list the details

Cardiovascular disease	Y / N	_____
Pulmonary disease	Y / N	_____
Stroke	Y / N	_____

### Lifestyle habits

If YES, how many times per week

Do you exercise regularly?	Y / N	_____
Do you smoke nicotine products?	Y / N	_____
Do you consume alcohol?	Y / N	_____
Do you consume tea or coffee?	Y / N	_____
Do you take recreational drugs?	Y / N	_____
Do you take supplements or ergogenic aids?	Y / N	_____



**APPENDIX C:**

**PHYSICAL ACTIVITY QUESTIONNAIRE**

## PHYSICAL ACTIVITY QUESTIONNAIRE

This questionnaire is designed to establish details of your physical activity routine and fitness level.

All information is strictly confidential.

### PERSONAL DETAILS

Name: \_\_\_\_\_ Contact Number: \_\_\_\_\_

Date of Birth: \_\_\_\_ / \_\_\_\_ / \_\_\_\_ Gender: M / F

### PHYSICAL ACTIVITY HISTORY

1. What level of fitness would you consider yourself?

- sedentary     mildly trained     moderately trained     very trained

2. What is the type of training that you most participate in?

- light aerobic     power     resistance     endurance     sport

3. What is the main exercise that you participate in? (ie – gym, run, basketball)

\_\_\_\_\_

4. What is the extent of your physical activity?

Frequency  Daily / almost daily

3-5 times per week

1-2 times per week

A few times per month

Less than once per month

Intensity  Sustained heavy breathing and perspiration (eg, running)

Intermittent heavy breathing and perspiration (eg, tennis / jogging)

Moderately heavy (eg, recreational sports)

Moderate (eg, volleyball / brisk walk)

Light (eg, walking)

Time  Over 30 minutes

20-30 minutes

10-20 minutes

Under 10 minutes

**APPENDIX D:**

**INSTRUCTIONS FOR EXERCISE PROTOCOL**

## **INSTRUCTIONS FOR EXERCISE PROTOCOL**

### **CONCENTRIC 1RM**

Step on the machine

Place body under the shoulder pads, going into a semi squat position, back straight

Place feet shoulder width apart

Slowly straighten knees until locked

Place right leg in the middle of the pad

Slowly lower to full dorsiflexion

Keep back straight and knee locked

Slowly lift heel into full plantar flexion

- five attempts at determining one RM

- two minutes rest is allowed between each repetition

### **Calculating the Eccentric weight load from the concentric**

= 60% of concentric 1RM

= 40% Ecc RM

## **EXERCISE PROTOCOL**

### **Warm Up**

Cycling for two minutes (50 revolutions per minute x one kg per minute)

5 minutes of stretching to follow, concentrating on the muscles of the TS

- soleus (20 secs each leg) x 2

- gastrocnemius (20 secs each leg) x 2

- quadriceps (20 secs each leg)

- hammy (20 secs each leg)

### **Protocol**

- three x 60 repetitions, or until the subject are unable to continue

- MVC test of the exercised and control leg after each set using the DAD and Amlab

- 10 minutes rest between each set

### **Calf Raise and Lower**

Step on the machine

Place body under the shoulder pads, going into a semi squat position, back straight

Step on with both feet, shoulder width apart

Slowly straighten knees until locked

Slowly lift heel into full plantar flexion

The arm is locked in place with the pin lock

Place feet shoulder width apart

Place body under the shoulder pads, back straight.

Place right leg in the middle of the pad

Lift to full plantarflexion and then slowly lower (for three seconds) to full dorsiflexion

Repeat from step 3

## **AFTER PROTOCOL**

Full testing protocol of the dependent variables

The subjects will be asked to refrain from any stretching or massage of the TS post-exercise

**APPENDIX E:**

**SUBJECT DATA SHEETS**

MVC

Right Average torque (Nm)

	0	1	1.5	2	3	24	48	72
1	55.91	53.61	50.38	50.09	43.90	41.64	37.31	47.24
2	81.32	56.18	50.26	54.37	69.41	77.19	86.43	95.78
3	84.00	78.66	77.43	66.78	63.50	67.43	66.22	81.67
4	76.87	69.47	59.08	57.90		60.75	60.97	72.28
5	41.75	41.45	42.53	40.20	41.25	32.88	32.09	43.54
6	80.80	73.92	48.23		39.79	48.89	56.36	75.76
7	67.90	56.18	65.37	63.74	65.85	65.69	57.46	66.76
8	55.01	44.25	45.67	42.56	44.69	55.93	50.89	45.61
9	52.13	44.63	40.00	37.00	39.70	44.58	44.06	48.92
10	53.68	48.16	44.96	45.85	47.91	48.78	54.20	53.04
11	106.23	103.72	98.40	96.80	97.65	93.95	99.91	101.54
av	68.69	60.93	56.57	55.53	55.37	57.97	58.72	66.56
SD	18.96	18.90	17.72	17.56	18.71	17.51	19.99	20.64
SEM	5.72	5.70	5.34	5.55	5.92	5.28	6.03	6.22

Right Average torque (Nm) - normalised

	0	1	1.5	2	3	24	48	72
1	100.00	95.89	90.10	89.59	78.52	74.48	66.72	84.50
2	100.00	69.09	61.80	66.85	85.36	94.92	106.28	117.78
3	100.00	93.64	92.17	79.49	75.60	80.28	78.83	97.23
4	100.00	90.36	76.85	75.32		79.02	79.31	94.02
5	100.00	99.29	101.86	96.28	98.79	78.75	76.85	104.28
6	100.00	91.49	59.69		49.24	60.51	69.75	93.76
7	100.00	82.73	96.26	93.87	96.98	96.74	84.62	98.31
8	100.00	80.44	83.02	77.36	81.23	101.66	92.51	82.91
9	100.00	85.62	76.73	70.98	76.16	85.51	84.51	93.83
10	100.00	89.72	83.76	85.43	89.26	90.88	100.98	98.81
11	100.00	97.64	92.63	91.12	91.92	88.44	94.05	95.58
av	100.00	88.72	83.17	82.63	82.31	84.65	84.95	96.45
% change		11.28	16.83	17.37	17.69	15.35	15.05	3.55
SD	0.00	8.82	13.52	10.09	14.26	11.69	12.45	9.34
SEM	0.00	2.66	4.08	3.19	4.51	3.52	3.75	2.82

Right PEAK torque (Nm)

	0	1	1.5	2	3	24	48	72
1	58.97	57.45	54.74	55.01	47.43	43.09	37.31	47.24
2	82.60	57.92	49.58	53.63	68.92	76.14	85.26	105.37
3	127.59	122.29	119.51	105.16	98.64	100.34	101.52	128.38
4	78.05	73.98	64.77	66.94		60.75	61.27	72.47
5	47.51	49.32	43.01	45.53	43.63	32.88	32.09	65.04
6	79.89	80.30	52.49		43.50	62.66	66.91	80.60
7	75.99	59.40	75.53	68.21	71.76	68.83	57.46	71.54
8	59.35	57.15	54.17	50.41	52.85	62.57	52.85	45.61
9	52.13	45.91	44.23	37.00	44.39	50.41	46.83	53.41
10	55.26	53.96	50.76	54.77	47.91	52.55	58.94	57.43
11	111.60	113.09	99.73	113.28	104.34	100.27	104.61	105.42
av	75.36	70.07	64.41	64.99	62.34	64.59	64.10	75.68
SD	25.23	25.61	24.53	25.09	23.00	21.31	23.96	26.93
SEM	7.61	7.72	7.40	7.94	7.27	6.42	7.23	8.12

Right PEAK torque (Nm) - normalised

	0	1	1.5	2	3	24	48	72
1	100.00	97.43	92.83	93.29	80.42	73.07	63.27	80.12
2	100.00	70.12	60.02	64.92	83.43	92.18	103.22	127.56
3	100.00	95.85	93.67	82.42	77.31	78.65	79.57	100.62
4	100.00	94.79	82.99	85.76		77.84	78.51	92.85
5	100.00	103.82	90.53	95.84	91.84	69.21	67.54	136.91
6	100.00	100.51	65.71		54.44	78.43	83.75	100.88
7	100.00	78.17	99.39	89.76	94.44	90.58	75.62	94.15
8	100.00	96.30	91.28	84.93	89.04	105.43	89.04	76.85
9	100.00	88.06	84.84	70.98	85.15	96.69	89.83	102.46
10	100.00	97.65	91.86	99.12	86.71	95.10	106.67	103.92
11	100.00	101.34	89.36	101.51	93.49	89.85	93.73	94.46
av	100.00	93.09	85.68	86.85	83.63	86.09	84.61	100.98
% change		6.91	14.32	13.15	16.37	13.91	15.39	-0.98
SD	0.00	10.36	12.15	11.80	11.66	11.29	13.62	17.84
SEM	0.00	3.12	3.66	3.73	3.69	3.41	4.11	5.38

**Right Soleus EMG (volts)**

	0	1	1.5	2	3	24	48	72
1	0.34	0.35	0.37	0.34	0.32	0.27	0.50	0.38
2	0.23	0.14	0.11	0.15	0.16	0.21	0.28	0.25
3	0.53	0.47	0.30	0.21	0.32	0.43	0.45	0.51
4	0.22	0.24	0.19	0.20		0.18	0.18	0.21
5	0.35	0.36	0.27	0.48	0.39	0.32	0.30	0.44
6	0.31	0.34	0.20		0.32	0.25	0.26	
7	0.46	0.22	0.18	0.32	0.42	0.43	0.44	0.43
8	0.16	0.13	0.16	0.13	0.10	0.11	0.14	0.14
9	0.18	0.17	0.17	0.17	0.17	0.12	0.14	0.09
10	0.32	0.26	0.27	0.20	0.31	0.25	0.31	0.32
11	0.50	0.42	0.39	0.40	0.40	0.40	0.46	0.47
av	0.33	0.28	0.24	0.26	0.29	0.27	0.32	0.32
SD	0.13	0.12	0.09	0.12	0.11	0.11	0.13	0.15
SEM	0.04	0.03	0.03	0.04	0.03	0.03	0.04	0.05

**Right gastroc EMG (volts)**

	Base	1	1.5	2	3	24	48	72
1	0.44	0.33	0.29	0.26	0.45	0.34	0.79	0.47
2	0.21	0.09	0.09	0.12	0.18	0.25	0.34	0.26
3	0.39	0.40	0.22	0.20	0.23	0.34	0.40	0.45
4	0.21	0.19	0.12	0.13		0.13	0.18	0.20
5	0.36	0.35	0.25	0.34		0.12	0.18	0.18
6	0.40	0.25	0.21		0.21	0.13	0.12	0.19
7	0.31	0.18	0.20	0.20	0.26	0.36	0.28	0.30
8								
9	0.16	0.14	0.08	0.14	0.19	0.08	0.10	0.10
10	0.31	0.25	0.26	0.21	0.26	0.12	0.17	0.17
11	0.26	0.25	0.23	0.25	0.25	0.23	0.29	0.22
av	0.31	0.24	0.19	0.21	0.25	0.21	0.29	0.25
SD	0.09	0.10	0.07	0.07	0.09	0.11	0.20	0.12
SEM	0.03	0.03	0.02	0.02	0.03	0.03	0.06	0.04

**Right Soleus EMG - normalised**

	0	1	1.5	2	3	24	48	72
1	100.00	101.76	108.61	98.53	93.64	77.98	147.95	112.13
2	100.00	60.23	47.31	65.17	68.51	91.15	122.35	109.43
3	100.00	87.72	56.61	38.90	60.04	79.61	84.91	95.82
4	100.00	108.80	86.73	94.75		81.33	83.64	95.37
5	100.00	102.36	77.24	136.83	110.95	90.75	84.32	123.32
6	100.00	112.43	64.99		104.36	83.32	85.71	
7	100.00	47.76	38.96	70.20	89.97	93.72	95.17	94.01
8	100.00	81.26	98.53	80.63	64.21	69.68	89.89	85.89
9	100.00	95.83	97.34	98.86	99.24	69.83	79.13	53.51
10	100.00	79.96	83.76	62.49	94.35	77.60	94.76	99.28
11	100.00	85.41	78.88	81.17	80.36	80.23	92.33	94.49
av	100.00	87.59	76.27	82.75	86.56	81.38	96.38	96.33
% change		12.41	23.73	17.25	13.44	18.62	3.62	3.67
SD	0.00	19.98	22.14	26.63	17.52	8.00	20.61	18.59
SEM	0.00	6.02	6.68	8.42	5.54	2.41	6.21	5.88

**Right gastroc EMG - normalised**

	Base	1	1.5	2	3	24	48	72
1	100.0	75.46	66.31	60.21	103.20	77.97	181.02	107.55
2	100.0	43.73	42.16	57.52	82.76	115.36	158.82	121.32
3	100.0	102.97	55.05	49.79	58.02	87.53	100.76	113.66
4	100.0	90.84	58.52	63.99		61.74	86.17	97.75
5	100.0	95.88	67.89	92.50		33.30	49.41	50.23
6	100.0	62.97	52.20		53.02	31.07	30.24	46.98
7	100.0	59.24	64.54	65.62	84.32	115.89	91.89	98.59
8								
9	100.0	84.43	47.13	88.93	118.65	46.72	62.09	63.52
10	100.0	79.44	83.96	67.33	83.10	39.94	55.97	53.61
11	100.0	95.69	86.17	95.05	95.94	87.69	111.04	84.39
av	100.0	79.06	62.39	71.22	84.88	69.72	92.72	83.76
% change		20.94	37.61	28.78	15.12	30.28	7.28	16.24
SD	0.00	18.89	14.51	16.59	21.85	32.01	47.90	28.05
SEM	0.00	5.97	4.59	5.53	7.73	10.12	15.15	8.87

**Left Average torque (Nm)**

	0	1	1.5	2	3	24	48	72
1	41.73	46.79	44.63	39.11	42.01	42.64	34.33	37.76
2	63.22	51.78	59.47	70.79	72.12	73.30	80.02	75.95
3	89.10	88.73	84.67	86.07	88.60	86.07	86.26	88.10
4	74.08	71.15	67.95	68.04		68.13	62.00	73.27
5	36.54	32.18	36.26	27.47	26.92	23.26		
6								
7	51.85	54.18	54.78	55.27	54.18	47.83	52.91	
8	44.51	41.82	41.29	44.14	47.06	41.42	43.03	42.25
9	50.33	48.04	48.20	48.90	43.19	57.53	57.03	52.79
10	57.87	48.12	47.95		48.71	54.48	50.38	51.71
11						98.54	93.34	91.33
av	56.58	53.64	53.91	54.97	52.85	59.32	62.15	64.15
SD	16.73	16.77	15.00	19.09	19.22	22.57	20.20	20.68
SEM	5.58	5.59	5.00	6.75	6.79	7.14	6.73	7.31

**Left Soleus EMG (volts)**

	0	1	1.5	2	3	24	48	72
1	0.45	0.32	0.33	0.32	0.33	0.32	0.29	0.38
2	0.16	0.08	0.19	0.22	0.21	0.24	0.24	0.26
3	0.42	0.43	0.42	0.43	0.42	0.39	0.33	0.42
4	0.29	0.20	0.23	0.27		0.20	0.18	0.20
5	0.21	0.19	0.18	0.24	0.20	0.22		
6								
7							0.42	
8		0.17	0.23			0.18	0.16	0.14
9	0.17	0.14	0.11	0.14	0.16	0.11		0.10
10	0.31	0.21	0.15		0.22	0.18	0.35	0.27
11						0.24	0.40	0.45
av	0.29	0.22	0.23	0.27	0.26	0.23	0.30	0.28
SD	0.12	0.11	0.10	0.10	0.10	0.08	0.10	0.13
SEM	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.05

**Left Average torque (Nm) - normalised**

	0	1	1.5	2	3	24	48	72
1	100.00	112.12	106.93	93.72	100.65	102.16	82.25	90.48
2	100.00	81.90	94.07	111.99	114.08	115.94	126.58	120.15
3	100.00	99.59	95.03	96.60	99.43	96.60	96.81	98.87
4	100.00	96.04	91.72	91.84		91.97	83.68	98.90
5	100.00	88.08	99.25	75.19	73.68	63.66		
6								
7	100.00	104.49	105.65	106.59	104.50	92.25	102.05	
8	100.00	93.97	92.78	99.18	105.74	93.07	96.69	94.94
9	100.00	95.45	95.76	97.16	85.81	114.30	113.32	104.89
10	100.00	83.16	82.86		84.17	94.14	87.07	89.36
11								
av	100.00	94.98	96.00	96.53	96.01	96.01	98.56	99.66
% change		5.02	4.00	3.47	3.99	3.99	1.44	0.34
SD	0.00	9.79	7.32	10.91	13.47	15.25	15.31	10.50
SEM	0.00	3.26	2.44	3.86	4.76	5.08	5.41	3.97

**Left Soleus EMG - normalised**

	0	1	1.5	2	3	24	48	72
1	100.00	71.53	73.75	70.80	73.38	70.87	63.72	83.26
2	100.00	48.16	118.85	132.99	130.94	147.75	150.20	162.70
3	100.00	101.26	98.42	101.65	99.05	91.96	78.72	99.05
4	100.00	68.52	79.77	91.70		68.18	61.59	67.84
5	100.00	94.80	85.37	115.28	95.45	107.97		
6								
7								
8								
9	100.00	83.67	67.53	82.67	95.62	65.74		57.77
10	100.00	66.67	47.85		72.26	57.10	112.58	87.10
11								
av	100.00	76.37	81.65	99.18	94.45	87.08	93.36	92.95
% change		23.63	18.35	0.82	5.55	12.92	6.64	7.05
SD	0.00	18.20	22.68	22.55	21.41	31.92	37.76	37.15
SEM	0.00	6.88	8.57	9.21	8.74	12.07	16.89	15.17



### EVOKED POTENTIALS

**Soleus Hmax:Mmax Ratio**

	0	2	3	24	48	72
1	0.59	0.55	0.47	0.55	0.52	0.58
2	0.29	0.20	0.18	0.18	0.17	0.33
3	0.53	0.13	0.32	0.35	0.44	0.37
4	0.37	0.44		0.38	0.36	0.36
5	0.54	0.44	0.64	0.54	0.51	0.51
6	0.33	0.26	0.29	0.26	0.29	0.54
7	0.47	0.49	0.47	0.47	0.67	0.52
8	0.38	0.22	0.39	0.23	0.27	0.40
9	0.26	0.13	0.11	0.12	0.10	0.25
10	0.26	0.13	0.14	0.32	0.34	0.23
11	0.53	0.51	0.49	0.50	0.48	0.48
av	0.41	0.32	0.35	0.35	0.38	0.42
SD	0.12	0.17	0.17	0.15	0.17	0.12
SEM	0.04	0.05	0.06	0.04	0.05	0.04

**Soleus Hmax:Mmax - Normalised**

	0	2	3	24	48	72
1	100.00	94.66	80.28	93.62	88.99	98.72
2	100.00	67.50	63.48	61.94	58.57	114.07
3	100.00	24.37	61.45	65.56	83.47	70.39
4	100.00	117.21		103.42	96.38	96.38
5	100.00	81.64	118.93	100.23	93.64	94.74
6	100.00	79.34	87.21	78.00	87.05	164.04
7	100.00	104.29	100.35	98.95	141.61	109.88
8	100.00	57.53	104.31	61.79	71.82	104.60
9	100.00	49.92	40.20	47.26	39.69	96.63
10	100.00	50.18	54.53	123.17	132.81	90.36
11	100.00	96.76	92.35	95.91	90.39	91.66
av	100.00	74.86	80.31	84.52	89.49	102.86
% change		25.14	19.69	15.48	10.51	-2.86
SD	0.00	27.81	24.89	23.09	29.07	23.28
SEM	0.00	8.38	7.87	6.96	8.76	7.02

**Soleus Peak H (volts)**

	0	2	3	24	48	72
1	6.63	6.37	4.43	6.30	5.40	5.43
2	1.83	1.00	1.37	1.20	1.23	2.23
3	5.83	1.43	3.67	3.50	4.50	4.13
4	2.60	2.93		1.50	2.37	2.47
5	4.93	4.10	5.00	5.43	4.50	5.03
6	2.97	1.76	1.77	1.80	1.47	2.77
7	2.53	2.87	2.07	3.33	3.83	3.20
8	2.53	1.26	1.93	1.33	1.60	2.37
9	1.83	0.73	0.57	0.67	0.67	1.37
10	2.53	0.93	1.17	2.63	3.20	2.27
11	5.40	4.63	4.37	4.67	4.23	4.40
av	3.60	2.55	2.64	2.94	3.00	3.24
SD	1.74	1.83	1.58	1.88	1.61	1.31
SEM	0.52	0.55	0.50	0.57	0.48	0.40

**Soleus Peak H - Normalised**

	0	2	3	24	48	72
1	100.00	96.08	66.82	95.02	81.45	81.90
2	100.00	54.64	71.86	65.57	67.21	121.86
3	100.00	24.53	62.95	60.03	77.19	70.84
4	100.00	112.69		57.69	91.15	95.00
5	100.00	83.16	101.42	110.14	91.28	102.03
6	100.00	59.26	59.60	60.61	49.49	93.27
7	100.00	113.44	81.82	131.62	151.38	126.48
8	100.00	49.80	76.28	52.57	63.24	93.68
9	100.00	39.89	31.15	36.61	36.61	74.86
10	100.00	36.76	46.25	103.95	126.48	89.72
11	100.00	85.74	80.93	86.48	78.33	81.48
av	100.00	68.73	68.21	78.21	83.08	93.74
% change		31.27	31.79	21.79	16.92	6.26
SD	0.00	31.03	19.77	29.15	32.67	17.66
SEM	0.00	9.36	6.25	8.79	9.85	5.33

99

### EVOKED POTENTIALS

#### Soleus Peak M (volts)

	0	2	3	24	48	72
1	11.33	11.50	9.43	11.50	10.37	9.40
2	6.30	5.10	7.43	6.67	7.23	6.73
3	11.03	11.10	11.30	10.10	10.20	11.10
4	7.00	6.73		5.20	6.17	6.90
5	9.10	9.27	7.76	10.00	8.87	9.80
6	8.97	6.70	6.13	6.97	5.10	5.10
7	5.36	5.83	4.37	7.13	5.73	6.17
8	6.70	5.80	4.90	5.70	5.90	6.00
9	6.97	5.57	5.40	5.40	6.43	5.40
10	9.87	7.23	8.37	8.33	9.40	9.80
11	10.27	9.10	9.00	9.27	8.90	9.13
av	8.45	7.63	7.41	7.84	7.66	7.78
SD	2.06	2.26	2.21	2.14	1.93	2.10
SEM	0.62	0.68	0.70	0.64	0.58	0.63

#### Gastroc Hmax:Mmax Ratio

	0	2	3	24	48	72
1	0.12	0.12	0.12	0.18	0.17	0.17
2	0.30	0.14	0.18	0.07	0.10	0.17
3	0.25	0.08	0.10	0.14	0.57	0.52
4	0.22	0.20		0.34	0.21	0.22
5	0.19	0.10	0.14	0.19		0.16
6	0.19	0.11	0.10	0.10	0.07	0.09
7	0.37	0.41	0.27		0.34	0.27
8	0.10	0.09	0.09	0.07	0.07	0.10
9	0.05	0.03	0.03	0.03	0.07	0.06
10	0.08	0.05	0.05	0.11		0.12
11	0.56	0.85	0.61	0.46	0.54	0.46
av	0.22	0.20	0.17	0.17	0.24	0.21
SD	0.15	0.24	0.17	0.13	0.20	0.15
SEM	0.04	0.07	0.05	0.04	0.07	0.05

#### Soleus Peak M - Normalised

	0	2	3	24	48	72
1	100.00	101.50	83.23	101.50	91.53	82.97
2	100.00	80.95	117.94	105.87	114.76	106.83
3	100.00	100.63	102.45	91.57	92.48	100.63
4	100.00	96.14		74.29	88.14	98.57
5	100.00	101.87	85.27	109.89	97.47	107.69
6	100.00	74.69	68.34	77.70	56.86	56.86
7	100.00	108.77	81.53	133.02	106.90	115.11
8	100.00	86.57	73.13	85.07	88.06	89.55
9	100.00	79.91	77.47	77.47	92.25	77.47
10	100.00	73.25	84.80	84.40	95.24	99.29
11	100.00	88.61	87.63	90.26	86.66	88.90
av	100.00	90.26	86.18	93.73	91.85	93.08
% change		9.74	13.82	6.27	8.15	6.92
SD	0.00	12.20	14.42	17.58	14.40	16.44
SEM	0.00	3.68	4.56	5.30	4.34	4.96

#### Gastroc Hmax:Mmax - Normalised

	0	2	3	24	48	72
1	100.00	98.44	97.58	153.52	143.45	147.44
2	100.00	47.13	58.70	22.06	33.52	55.15
3	100.00	31.73	40.57	56.80	230.85	210.59
4	100.00	87.17		152.30	93.40	97.96
5	100.00	52.62	73.09	100.70		85.27
6	100.00	57.29	51.91	53.62	36.42	45.81
7	100.00	110.45	73.00		93.04	74.12
8	100.00	88.14	89.44	69.97	64.19	98.22
9	100.00	51.62	48.35	63.55	126.04	111.84
10	100.00	58.98	61.64	133.54		149.34
11	100.00	152.35	110.08	81.58	96.39	82.17
av	100.00	75.99	70.44	88.76	101.92	105.26
% change		24.01	29.56	11.24	-1.92	-5.26
SD	0.00	35.17	22.66	44.88	60.73	47.86
SEM	0.00	10.60	7.17	14.19	20.24	14.43

### EVOKED POTENTIALS

**Gastroc Peak H (volts)**

	0	2	3	24	48	72
1	1.60	1.40	1.33	1.23	1.27	1.73
2	0.87	0.41	0.50	0.43	0.63	1.07
3	2.67	0.80	0.97	1.40	4.60	4.67
4	0.80	0.67		1.00	0.90	0.90
5	1.93	1.30	1.57	1.93		1.70
6	1.30	0.67	0.70	0.67	0.43	0.37
7	1.10	0.90	0.83		1.63	1.40
8	1.00	0.70	0.90	0.87	0.87	1.17
9	0.63	0.33	0.37	0.47	0.87	0.87
10	0.77	0.40	0.40	0.80		1.17
11	1.97	2.10	1.80	1.73	1.63	1.77
av	1.33	0.88	0.94	1.05	1.43	1.53
SD	0.64	0.53	0.49	0.51	1.26	1.12
SEM	0.19	0.16	0.16	0.16	0.42	0.34

**Gastroc Peak H - Normalised**

	0	2	3	24	48	72
1	100.00	87.50	83.13	76.88	79.38	108.13
2	100.00	47.13	57.47	49.43	72.41	122.99
3	100.00	29.96	36.33	52.43	172.28	174.91
4	100.00	83.75		125.00	112.50	112.50
5	100.00	67.36	81.35	100.00		88.08
6	100.00	51.54	53.85	51.54	33.08	28.46
7	100.00	81.82	75.45		148.18	127.27
8	100.00	70.00	90.00	87.00	87.00	117.00
9	100.00	52.38	58.73	74.60	138.10	138.10
10	100.00	51.95	51.95	103.90		151.95
11	100.00	106.60	91.37	87.82	82.74	89.85
av	100.00	66.36	67.96	80.86	102.85	114.48
% change		33.64	32.04	19.14	-2.85	-14.48
SD	0.00	22.22	18.71	25.04	43.61	38.21
SEM	0.00	6.70	5.92	7.92	14.54	11.52

**Gastroc Peak M (volts)**

	0	2	3	24	48	72
1	13.50	12.00	11.50	6.76	7.47	9.90
2	6.80	6.80	6.80	6.43	6.20	6.40
3	10.80	10.20	9.67	9.97	8.06	8.97
4	3.57	3.43		2.93	4.30	4.10
5	10.00	12.80	11.13	9.93		10.33
6	6.97	6.27	7.23	6.70	6.33	4.33
7	2.97	2.20	3.07	3.97	3.70	4.10
8	9.57	7.60	9.63	11.90	12.97	11.40
9	11.50	11.67	13.97	13.50	12.60	14.20
10	9.73	8.57	8.20	7.57	6.90	9.90
11	3.53	2.47	2.93	3.80	3.03	3.86
av	8.09	7.64	8.41	7.59	7.16	7.95
SD	3.57	3.82	3.55	3.42	3.39	3.57
SEM	1.08	1.15	1.12	1.03	1.07	1.08

**Gastroc Peak M - Normalised**

	0	2	3	24	48	72
1	100.00	88.89	85.19	50.07	55.33	73.33
2	100.00	100.00	100.00	94.56	91.18	94.12
3	100.00	94.44	89.54	92.31	74.63	83.06
4	100.00	96.08		82.07	120.45	114.85
5	100.00	128.00	111.30	99.30		103.30
6	100.00	89.96	103.73	96.13	90.82	62.12
7	100.00	74.07	103.37	133.67	124.58	138.05
8	100.00	79.41	100.63	124.35	135.53	119.12
9	100.00	101.48	121.48	117.39	109.57	123.48
10	100.00	88.08	84.28	77.80	70.91	101.75
11	100.00	69.97	83.00	107.65	85.84	109.35
av	100.00	91.85	98.25	97.75	95.88	102.05
% change		8.15	1.75	2.25	4.12	-2.05
SD	0.00	15.70	12.69	23.33	25.94	22.60
SEM	0.00	4.74	4.01	7.03	8.20	6.81

### CONTRACTILE PROPERTIES

#### peak twitch (volts)

	0	2	3	24	48	72
1	35.22	25.59	34.63	14.73	34.34	29.78
2	42.90	30.00	36.70	34.76	32.01	39.48
3	54.29	34.12	36.88	44.84	52.39	55.62
4	46.79	43.26		43.75	40.11	45.67
5	23.72	16.35	21.40	19.55	20.90	26.65
6	32.59	25.06	20.70	26.06	23.67	35.43
7	30.50	32.96	29.73	30.37	30.83	
8	24.90	17.35	19.03	33.40	23.25	32.75
9	28.93	19.47	21.60	29.93	27.15	27.10
10	25.00	18.36	24.47	34.07	32.63	29.87
11	26.94	28.92	30.93	35.53	37.33	37.88
av	33.80	26.49	27.61	31.54	32.24	36.02
SD	10.06	8.39	6.54	7.17	8.97	8.85
SEM	3.03	2.53	2.07	2.16	2.70	2.80

#### peak twitch (Nm)

	0	2	3	24	48	72
1	9.67	7.03	9.51	4.05	9.43	8.18
2	11.79	8.24	10.08	9.55	8.79	10.85
3	14.91	9.37	10.13	12.32	14.39	15.28
4	12.86	11.88		12.02	11.02	12.55
5	6.52	4.49	5.88	5.37	5.74	7.32
6	8.95	6.88	5.69	7.16	6.50	9.73
7	8.38	9.05	8.17	8.34	8.47	
8	6.84	4.77	5.23	9.18	6.39	9.00
9	7.95	5.35	5.93	8.22	7.46	7.45
10	6.87	5.04	6.72	9.36	8.97	8.21
11	7.40	7.95	8.50	9.76	10.26	10.41
av	9.29	7.28	7.58	8.67	8.86	9.90
SD	2.76	2.30	1.80	1.97	2.46	2.43
SEM	0.83	0.69	0.57	0.59	0.74	0.77

#### peak twitch (volts)- Normalised

	0	2	3	24	48	72
1	100.00	72.67	98.34	41.83	97.52	84.57
2	100.00	69.93	85.55	81.03	74.62	92.04
3	100.00	62.85	67.94	82.60	96.51	102.45
4	100.00	92.45		93.49	85.72	97.59
5	100.00	68.93	90.22	82.42	88.11	112.35
6	100.00	76.88	63.53	79.97	72.62	108.71
7	100.00	108.07	97.49	99.56	101.09	
8	100.00	69.68	76.44	134.14	93.37	131.53
9	100.00	67.28	74.65	103.46	93.84	93.66
10	100.00	73.43	97.88	136.28	130.55	119.48
11	100.00	107.35	114.82	131.90	138.58	140.62
av	100.00	79.05	86.69	96.97	97.50	108.30
% change		20.95	13.31	3.03	2.50	-8.30
SD	0.00	16.04	16.06	28.65	20.48	18.00
SEM	0.00	4.84	5.08	8.64	6.17	5.69

#### peak twitch (Nm) - Normalised

	0	2	3	24	48	72
1	100.00	72.67	98.34	41.83	97.52	84.57
2	100.00	69.93	85.55	81.03	74.62	92.04
3	100.00	62.85	67.94	82.60	96.51	102.45
4	100.00	92.45		93.49	85.72	97.59
5	100.00	68.93	90.22	82.42	88.11	112.35
6	100.00	76.88	63.53	79.97	72.62	108.71
7	100.00	108.07	97.49	99.56	101.09	
8	100.00	69.68	76.44	134.14	93.37	131.53
9	100.00	67.28	74.65	103.46	93.84	93.66
10	100.00	73.43	97.88	136.28	130.55	119.48
11	100.00	107.35	114.82	131.90	138.58	140.62
av	100.00	79.05	86.69	96.97	97.50	108.30
% change		20.95	13.31	3.03	2.50	-8.30
SD	0.00	16.04	16.06	28.65	20.48	18.00
SEM	0.00	4.84	5.08	8.64	6.17	5.69

### CONTRACTILE PROPERTIES

TTP						
	0	2	3	24	48	72
1	117	117	115	137	135	115
2	129	119	139	138	142	140
3	138	115	129	110	140	138
4	130	118		132	141	123
5	129	131	140	139	138	144
6	114	107	121	117	128	127
7	133	105	133	129	138	
8	124	138	136	136	137	138
9	137	124	121	117	118	137
10	132	118	126	121	156	122
11	132	136	138	129	144	133
av	129	121	130	128	138	132
SD	6.47	10.70	7.00	9.37	9.48	7.28
SEM	1.95	3.23	2.21	2.83	2.86	2.30

HRT						
	0	2	3	24	48	72
1	44	73	23	53	62	56
2	61	47	54	58	55	72
3	42	34	31	41	37	37
4	86	59		79	95	47
5	76	42	73	82	68	57
6	63	44	47	55	54	42
7	93	86	88	86	78	
8	87	79	80	81	86	76
9	76	34	63	84	40	59
10	57	29	55	64	74	69
11	54	47	46	56	52	49
av	67	52	56	67	64	56
SD	15.87	17.98	17.11	14.90	18.34	12.93
SEM	4.78	5.42	5.41	4.49	5.53	4.09

TTP - normalised						
	0	2	3	24	48	72
1	100.00	100.28	98.29	117.09	115.10	98.29
2	100.00	92.25	107.75	107.24	110.08	108.53
3	100.00	83.27	93.45	80.00	101.45	100.36
4	100.00	90.77		101.15	108.46	94.36
5	100.00	101.16	108.14	107.36	106.98	111.24
6	100.00	93.57	106.14	102.92	112.28	111.40
7	100.00	78.50	99.50	96.50	103.50	
8	100.00	110.59	109.38	108.98	109.79	110.72
9	100.00	90.53	88.11	85.19	85.92	99.51
10	100.00	89.39	95.45	91.67	118.28	92.68
11	100.00	103.54	104.56	97.97	109.62	101.27
av	100.00	93.99	101.08	99.64	107.41	102.84
% change		6.01	-1.08	0.36	-7.41	-2.84
SD	0.00	9.27	7.22	10.92	8.56	7.10
SEM	0.00	2.80	2.28	3.29	2.58	2.25

HRT - normalised						
	0	2	3	24	48	72
1	100.00	164.66	51.88	120.30	139.85	125.56
2	100.00	77.05	88.52	95.08	90.16	118.03
3	100.00	81.60	75.20	98.40	89.60	89.60
4	100.00	68.60		91.28	110.47	54.65
5	100.00	55.26	96.05	107.89	89.47	75.00
6	100.00	70.74	75.00	87.23	85.64	67.02
7	100.00	92.47	94.62	92.83	84.23	
8	100.00	90.23	91.95	93.10	98.28	87.36
9	100.00	45.18	82.89	110.09	52.19	77.19
10	100.00	51.76	96.47	113.53	130.00	121.76
11	100.00	87.65	84.57	103.70	96.91	90.74
av	100.00	80.47	83.72	101.22	96.98	90.69
% change		19.53	16.28	-1.22	3.02	9.31
SD	0.00	32.14	13.70	10.59	23.59	24.10
SEM	0.00	9.69	4.33	3.19	7.11	7.62

### TENDON TAP

#### s - peak to peak

	0	2	3	24	48	72
1	2.22	2.39	2.27	2.34	2.06	1.40
2	1.12	1.25	1.01	1.18	1.27	1.19
3	3.33	2.66	2.77	3.22	2.52	3.20
4	1.91	1.93		1.63	2.09	2.22
5	3.68	2.70	3.71	4.18	3.30	3.79
6	2.41		1.87	1.82	1.53	2.02
7	2.36	2.79	2.19	3.21	2.78	0.00
8	0.53	0.47	0.61	0.60	0.61	0.60
9	1.73	1.53	1.50	1.41	1.40	1.37
10	2.38	1.33	2.13	2.51	2.37	2.60
11	2.50	2.35	2.54	1.68	2.18	2.10
av	2.20	1.94	2.06	2.16	2.01	1.86
SD	0.89	0.77	0.89	1.05	0.76	1.11
SEM	0.27	0.24	0.28	0.32	0.23	0.33

#### ic - peak to peak

	0	2	3	24	48	72
1	0.57	0.51	0.60	0.69	0.77	0.54
2	0.35	0.38	0.47	0.46	0.49	0.49
3	1.31	1.35	1.09	1.08	1.18	1.30
4	0.76	0.69		0.86	0.84	0.87
5	0.96	0.78	1.01	1.00	0.89	0.94
6	1.69		0.61	0.77	0.87	1.02
7	0.98	1.11	1.02	1.69	1.43	
8	0.23	0.30	0.27	0.31	0.23	0.27
9	0.51	0.31	0.41	0.47	0.30	0.41
10	0.77	0.58	0.89	1.02	0.94	1.10
11	0.94	1.03	1.13	0.67	0.81	0.73
av	0.82	0.70	0.75	0.82	0.80	0.77
SD	0.42	0.36	0.31	0.38	0.35	0.33
SEM	0.13	0.11	0.10	0.11	0.11	0.11

#### Soleus - peak to peak - Normalised

	0	2	3	24	48	72
1	100.00	107.59	101.91	105.12	92.74	62.77
2	100.00	111.36	89.98	105.46	112.84	106.19
3	100.00	80.11	83.20	96.95	75.68	96.34
4	100.00	100.98		85.25	109.57	116.26
5	100.00	73.47	100.85	113.61	89.66	103.06
6	100.00		77.65	75.52	63.43	83.92
7	100.00	117.99	92.59	135.98	117.81	
8	100.00	88.68	115.09	113.21	115.09	113.21
9	100.00	88.44	86.71	81.50	80.92	79.19
10	100.00	55.67	89.64	105.64	99.44	109.24
11	100.00	94.00	101.50	67.00	87.00	84.00
av	100.00	91.83	93.91	98.66	94.93	95.42
% change		8.17	6.09	1.34	5.07	4.58
SD	0.00	18.87	10.98	19.95	17.72	17.35
SEM	0.00	5.97	3.47	6.01	5.34	5.49

#### Gastroc - peak to peak - Normalised

	0	2	3	24	48	72
1	100.00	90.07	106.40	121.63	136.64	94.70
2	100.00	108.54	132.38	129.89	139.50	139.50
3	100.00	102.86	83.05	82.10	89.62	98.67
4	100.00	91.58		113.37	111.39	115.02
5	100.00	81.30	104.55	104.29	92.86	97.14
6	100.00		36.07	45.68	51.29	60.46
7	100.00	114.10	104.49	173.08	147.01	
8	100.00	130.43	117.39	134.78	100.00	117.39
9	100.00	60.78	80.39	92.16	58.82	80.39
10	100.00	74.68	114.94	132.03	122.08	142.86
11	100.00	109.31	119.68	71.41	85.64	77.66
av	100.00	96.37	99.93	109.13	103.17	102.38
% change		3.63	0.07	-9.13	-3.17	-2.38
SD	0.00	20.71	27.49	35.17	31.71	26.57
SEM	0.00	6.55	8.69	10.60	9.56	8.40

Torque (Nm)							
	0	2	3	24	48	72	
1	3.67	2.83	4.14	1.49	3.51	3.11	
2	3.76	3.63	3.73	3.22	3.30	3.93	
3	7.22	4.63	5.46	5.49	5.76	6.38	
4	5.56	5.68		5.13	5.36	6.15	
5	4.39	2.91	4.21	3.73	3.14	4.76	
6	4.15		3.85	3.18	3.04	4.40	
7	3.84	4.48	4.29	4.80	3.92		
8	2.77	3.32	3.82	2.88	3.08	3.05	
9	2.80	2.66	2.77	3.32	2.61	2.91	
10	3.68	2.61	4.11	4.42	3.16	3.57	
11	4.48	4.89	5.66	5.10	5.92	6.18	
av	4.21	3.76	4.20	3.89	3.89	4.44	
SD	1.26	1.08	0.83	1.21	1.20	1.37	
SEM	0.38	0.34	0.26	0.37	0.36	0.43	

### CREATINE KINASE

CK					
	0	3	24	48	72
1	158.0	110.0	84.0	299.0	476.0
2	245.0	353.0	335.0	157.0	130.0
3	101.0	118.0	85.3	432.0	159.0
4	115.0	109.0	136.0		126.0
5	130.0	196.0	320.0	213.0	203.0
6	74.7	352.0	279.0	307.0	106.0
7	58.4	80.2	82.4	106.0	85.0
8	61.6	80.0	140.0	104.0	
9	143.0	201.0	173.0	343.0	394.0
10	122.0	130.0	163.0	106.0	98.0
11	155.0	166.0	299.0	501.0	133.0
av	124.0	172.3	190.6	256.8	191.0
SD	53.3	98.0	95.0	142.5	134.2
SEM	16.1	29.5	29.8	45.1	42.4

Torque (Nm)- normalised							
	0	2	3	24	48	72	
1	100.00	77.28	113.07	40.66	95.64	84.78	
2	100.00	96.53	99.18	85.74	87.93	104.69	
3	100.00	64.13	75.69	76.07	79.78	88.39	
4	100.00	102.17		92.22	96.30	110.53	
5	100.00	66.25	95.93	85.12	71.57	108.54	
6	100.00		92.80	76.59	73.28	105.87	
7	100.00	116.92	111.91	125.07	102.27		
8	100.00	119.80	137.62	103.96	110.89	109.90	
9	100.00	95.10	99.02	118.63	93.14	103.92	
10	100.00	70.90	111.57	120.02	85.82	97.01	
11	100.00	109.04	126.28	113.87	132.11	138.01	
av	100.00	91.81	###	94.36	93.52	###	
% change		8.19	-6.31	5.64	6.48	-5.16	
SD	0.00	20.87	17.69	25.22	17.46	14.56	
SEM	0.00	6.60	5.59	7.60	5.27	4.60	

CK - Normalised					
	0	3	24	48	72
1	100.0	69.6	53.2	189.2	301.3
2	100.0	144.1	136.7	64.1	53.1
3	100.0	116.8	84.5	427.7	157.4
4	100.0	94.8	118.3		109.6
5	100.0	150.8	246.2	163.8	156.2
6	100.0	471.2	373.5	411.0	141.9
7	100.0	137.3	141.1	181.5	145.5
8	100.0	129.9	227.3	168.8	
9	100.0	140.6	121.0	239.9	275.5
10	100.0	106.6	133.6	86.9	80.3
11	100.0	107.1	192.9	323.2	85.8
av	100.0	151.7	166.2	225.6	150.7
SD	0.0	108.7	89.5	125.0	80.8
SEM	0.0	32.8	27.0	39.5	25.6

**APPENDIX F:**

**Correlation Matrix**



Correlations of Normalised Values

	SOL H	SOL M	MG H:M	MG H	MG M	TORQUE	SOL EMG	GAS EMG	TTP	HRT	TPT	SOL T	GAS T	T TORQUE
SOL H:M	0.83**	0.05	0.26*	0.33**	0.06	0.27*	0.31**	-0.05	0.39**	0.27*	0.40**	0.29*	0.16	0.22*
SOL H		0.55**	0.29*	0.48**	0.20	0.44**	0.36**	0.15	0.29*	0.37**	0.37**	0.54**	0.41**	0.18
SOL M			0.13	0.33**	0.45**	0.45**	0.23*	0.36**	-0.01	0.24*	0.06	0.54**	0.57**	-0.07
MG H:M				0.78**	0.44*	0.06	0.10	0.17	0.06	0.29*	0.29*	0.07	0.21	0.03
MG H					0.10	0.31**	0.16	0.23	0.07	0.22*	0.42**	0.26*	0.30*	0.18
MG M						0.30**	0.16	0.33**	0.06	-0.06	-0.04	0.38**	0.37**	0.13
TORQUE							0.31**	0.23*	0.12	0.14	0.46**	0.32**	0.33**	0.28*
SOL EMG								0.56**	0.34**	0.21	0.08	0.11	0.08	0.00
GAS EMG									0.17	0.15	0.07	0.23	0.41**	0.19
TTP										0.28**	0.08	0.13	0.20	-0.02
HRT											0.18	0.23	0.36**	-0.05
TPT												0.18	0.20	0.63**
SOL T													0.69**	0.32**
GAS T														0.24*
T TORQUE														0.50

(\* p < 0.05, \*\* p < 0.01)

**APPENDIX G:**

**PILOT STUDY DATA SHEETS**

**Soleus Peak H (volts)**

	1	2	3	4	5	6	7	8	9	10	11	12	av
Base	4.60	0.47	1.70	2.65	4.49	2.27	2.27	4.47	3.94	2.40			2.93
1	4.63	1.77	1.33	1.93	3.74	1.95	1.95	5.24	3.15	1.55			2.72
diff	-0.03	-1.30	0.37	0.72	0.75	0.32	0.32	-0.77	0.79	0.85	0.00	0.00	0.17

**Soleus Peak M (volts)**

	1	2	3	4	5	6	7	8	9	10	11	12	av
Base	8.46	8.20	6.93	4.50	8.20	6.50	4.89	6.87	8.33	5.95			6.88
1	8.27	9.23	2.47	4.30	8.77	7.12	5.05	7.92	6.09	6.49			6.57
diff	0.19	-1.03	4.46	0.20	-0.57	-0.62	-0.16	-1.05	2.24	-0.54			0.31

**Soleus Hmax:Mmax Ratio**

	1	2	3	4	5	6	7	8	9	10	11	12	av
Base	0.54	0.06	0.25	0.59	0.55	0.63	0.46	0.65	0.47	0.40			0.46
1	0.56	0.19	0.25	0.45	0.43	0.60	0.39	0.66	0.52	0.24			0.43
diff	-0.02	-0.13	0.00	0.14	0.12	0.04	0.08	-0.01	-0.04	0.16			0.03

**Gastroc Peak H (volts)**

	1	2	3	4	5	6	7	8	9	10	11	12	av
Base	2.16	0.70	0.77	0.87	1.50	1.45	0.97	1.89	1.25	0.77			1.23
1	1.97	0.37	0.60	1.35	1.09	0.77	1.00	1.98	1.39	0.67			1.12
diff	0.19	0.33	0.17	-0.48	0.41	0.68	-0.03	-0.09	-0.14	0.10			0.11

**Gastroc Peak M (volts)**

	1	2	3	4	5	6	7	8	9	10	11	12	av
Base	2.67	4.70	6.53	2.44	4.39	7.92	3.63	3.64	10.29	5.54			5.18
1	4.13	8.70	6.50	2.72	4.03	6.45	2.75	3.42	9.65	5.68			5.40
diff	-1.46	-4.00	0.03	-0.28	0.36	1.47	0.88	0.22	0.64	-0.14			-0.2

**Gastroc Hmax:Mmax Ratio**

	1	2	3	4	5	6	7	8	9	10	11	12	av
Base	0.41	0.15	0.12	0.36	0.34	0.18	0.50	0.52	0.32	0.14			0.30
1	0.48	0.03	0.09	0.50	0.27	0.12	0.36	0.58	0.31	0.12			0.29
diff	-0.07	0.12	0.03	-0.14	0.07	0.06	0.13	-0.06	0.01	0.02			0.02

**Time to peak of evoked twitch**

	1	2	3	4	5	6	7	8	9	10	11	12	av
Base	105	74	127	130	127	108	126	129	128		110	126	117
1	119	127	123	117	122	111	114	129	132		116	107	120
diff	-14	-53	4	13	5	-2	12	0	-4	0	-6	19	-2

**HRT of evoked twitch**

	1	2	3	4	5	6	7	8	9	10	11	12	av
Base	76	123	69	83	47	60	77	96	65		74	81	77
1	80	86	74	107	75	65	70	110	65		48	76	78
diff	-4	37	-5	-24	-27	-5	6	-15	0		26	5	0

**TPT of evoked twitch**

	1	2	3	4	5	6	7	8	9	10	11	12	av
Base	10	5	11	10	5	15	7	11	18		11	7	10
1	10	5	13	9	8	14	9	13	10		15	10	11
diff	0	0	-1	2	-3	1	-2	-2	8		-4	-3	0

**Av Soleus T-wave**

base	1.02	0.50	0.72	1.10	0.69	3.26	1.62	3.68	1.99	2.35	1.86	1.73	1.71
1	1.24	1.34	0.50	0.79	1.72	2.69	1.65	4.65	2.17	1.92	2.49	2.03	1.93
diff	-0.23	-0.84	0.22	0.30	-1.03	0.57	-0.03	-0.97	-0.18	0.43	-0.63	-0.31	-0.22

**Av Gastroc T-wave**

base	0.58	0.96	0.18	0.52	0.31	1.32	1.08	1.56	0.57	0.85	0.35	1.36	0.80
1	0.54	0.30	0.16	0.54	0.57	1.04	1.08	1.90	0.70	0.69	0.39	1.18	0.76
diff	0.04	0.66	0.02	-0.02	-0.26	0.29	0.00	-0.35	-0.13	0.17	-0.05	0.17	0.05

**T-reflex torque**

base	4.80	2.70	4.05	3.57	2.20	8.50	4.68	6.13	6.36	4.28	4.18	4.18	4.64
1	5.52	2.72	4.40	3.77	1.92	7.23	4.57	8.32	8.70	5.69	4.85	4.85	5.21
diff	-0.72	-0.02	-0.34	-0.20	0.28	1.28	0.10	-2.19	-2.34	-1.41	-0.66	-0.66	-0.57

**MVC Volts**

	1	2	3	4	5	6	7	8	9	10	11	12	av
Base	262	209	54	284	119	318	178	160	142	156	193	218	191
1	315	189	118	279	159	255	166	110	215	163	220	281	206
diff	-53	20	-64	5	-41	63	12	51	-73	-7	-27	-63	-15

**Soleus MVC EMG**

	1	2	3	4	5	6	7	8	9	10	11	12	av
Base	0.27	0.18	0.06	0.29	0.18	0.28	0.34	0.18	0.17	0.18	0.23	0.25	0.22
1	0.42	0.20	0.12	0.28	0.24	0.17	0.22	0.15	0.13	0.11	0.27	0.30	0.22
diff	-0.15	-0.03	-0.07	0.01	-0.06	0.10	0.12	0.03	0.04	0.07	-0.04	-0.05	0.00

**Gastroc MVC EMG**

	1	2	3	4	5	6	7	8	9	10	11	12	av
Base	0.18	0.26	0.24	0.27	0.07	0.18	0.30	0.11	0.07	0.28	0.08	0.10	0.18
1	0.18	0.09	0.22	0.28	0.06	0.09	0.15	0.07	0.19	0.32	0.20	0.15	0.17
diff	0.00	0.17	0.01	-0.01	0.00	0.10	0.15	0.04	-0.12	-0.04	-0.12	-0.04	0.01