

**THE EFFECTS OF EXERCISE-INDUCED MUSCLE DAMAGE
ON THE HUMAN RESPONSE TO DYNAMIC EXERCISE**

Submitted by Rosemary C. Davies to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Sport and Health Sciences (May, 2010).

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Abstract

Exercise-induced muscle damage (EIMD) is a commonly experienced phenomenon, yet its effect on the human response to dynamic exercise is poorly understood. Therefore the intention of this thesis was to provide empirical evidence to advance the scientific knowledge and understanding of the phenomenon of EIMD; principally by investigating the physiological, perceived exertion and metabolic responses to the performance of dynamic exercise with EIMD. The eccentric, muscle-damaging exercise protocol employed for all four studies involved participants completing 100 squats performed as 10 sets of 10 repetitions with the load on the bar corresponding to 70% of the individual's body mass. Measures of markers of muscle damage were taken before and after the eccentric exercise protocol in each of the four studies. The markers used were plasma creatine kinase activity, isokinetic peak torque and perceived muscle soreness. Cycling rather than running was used as the dynamic exercise mode in studies 1, 2 and 4 in order to avoid the confounding influence of alterations in gait subsequent to EIMD. The dynamic exercise in study 3 was performed inside a whole body scanner and was therefore limited to knee extension and flexion.

These four studies have provided novel insights into the influence of eccentric, muscle-damaging exercise on the human response to the performance of dynamic exercise. We have demonstrated for the first time that following EIMD, the enhanced ventilatory response to dynamic exercise is provoked by stimuli unrelated to the blood lactate response, and that this enhanced ventilation may provide an important cue to inform the perception of effort. Furthermore, we have shown that the reduced time to exhaustion observed following EIMD is associated with an elevated perception of exertion and increases in $[Pi]$ during dynamic exercise. Finally, we have demonstrated that the $\dot{V}O_2$ kinetic response is unaltered during the transition to high intensity dynamic exercise. Changes in $[HHb]$ kinetics indicate that compensatory mechanisms act to preserve blood-myocyte O_2 flux in the face of microvascular dysfunction, resulting in the unaltered $\dot{V}O_2$ observed across the rest-to-exercise transition.

Acknowledgments

Firstly, I would like to thank Dr Ann Rowlands for her support and expert guidance. She has been an exceptional supervisor and valued mentor. From assisting me on my first day of data collection to proof reading the final draft of this thesis, she has always been there for me. I cannot thank her enough for her help in transforming me from a disillusioned secondary school teacher to an enthusiastic independent researcher. I would also like to thank Professor Roger Eston, my second supervisor, who has supported me throughout my time at Exeter. His infectious enthusiasm and rare ability to encourage curiosity have been inspirational.

In addition to my supervisory team I have been privileged to work under the expert guidance of several other academics, notably Professors Andy Jones and David Poole. They have been generous in sharing their expertise and encouraging me throughout the course of my studies. I am also indebted to numerous other members of the academic, technical and administrative staff, as well as other research students at the School of Sport and Health Sciences for their assistance in the completion of this thesis. I would like to acknowledge the efforts of the many volunteers, or victims as they have asked to be called, who participated in the various studies. Without their willing cooperation none of this would have been possible.

Finally, I would like to thank my family and friends who have who have stood by me over the last few years. In particular, I would like to acknowledge the unswerving support of my parents, Liliias and Jim Davies. This is for you.

List of contents

List of Figures	8
List of Tables	11
Chapter 1 Introduction	
1.1 Introduction	13
1.2 Mechanisms of muscle damage	13
1.3 Muscle function following eccentric exercise	16
1.4 Dynamic exercise	17
1.5 Summary	17
Chapter 2 Review of literature	
2.1 Introduction	24
2.2 Changes in skeletal muscle morphology	24
2.3 Indirect markers of muscle damage	26
2.3.1 <i>Muscle protein efflux</i>	27
2.3.2 <i>Calcium homeostasis</i>	28
2.3.3 <i>The inflammatory response</i>	29
2.3.4 <i>Impaired metabolism</i>	30
2.3.5 <i>Delayed onset muscle soreness (DOMS)</i>	32
2.3.6 <i>Changes in strength</i>	34
2.4 Muscle function following exercise-induced muscle damage	35
2.4.1 <i>Changes in optimal muscle length</i>	36
2.4.2 <i>Low-frequency fatigue</i>	37
2.4.3 <i>Alterations to neural control</i>	38
2.5 The Repeated Bout Effect	41
2.6 Dynamic muscle function following exercise-induced muscle damage	44
2.6.1 <i>Wingate 30 s cycle test</i>	44
2.6.2 <i>Sprint performance</i>	45
2.6.3 <i>Vertical jump tests</i>	46
2.6.4 <i>Endurance exercise</i>	48

2.7	Human response to dynamic exercise	49
	2.7.1 <i>Oxygen uptake</i>	50
	2.7.2 <i>Oxygen uptake kinetics and muscle oxygenation</i>	52
	2.7.3 <i>Metabolic responses to dynamic exercise</i>	54
	2.7.4 <i>Ventilatory responses to dynamic exercise</i>	57
	2.7.5 <i>Perception of effort during dynamic exercise</i>	60
2.8	Conclusions	61

Chapter 3 Common Methods

3.1	Introduction	64
3.2	Familiarisation	64
3.3	Eccentric, muscle-damaging exercise protocol	65
3.4	Markers of muscle damage	67
	3.4.1 <i>Creatine Kinase activity</i>	67
	3.4.2 <i>Perceived muscle soreness</i>	68
	3.4.3 <i>Isokinetic peak torque</i>	68

Chapter 4 The effect of exercise-induced muscle damage on ventilatory and perceived exertion responses to moderate and severe intensity cycle exercise

4.1	Abstract	72
4.2	Introduction	73
4.3	Methods	76
4.4	Results	82
4.5	Discussion	88
4.6	Conclusion	93

Chapter 5: The effect of eccentric exercise-induced muscle damage on the gas exchange threshold

5.1	Abstract	95
5.2	Introduction	96
5.3	Methods	98
5.4	Results	103

5.5	Discussion	109
5.6	Conclusion	114

Chapter 6: The ³¹P-MRS metabolic response to incremental exercise following eccentric, muscle-damaging exercise

6.1	Abstract	116
6.2	Introduction	117
6.3	Methods	120
6.4	Results	125
6.5	Discussion	131
6.6	Conclusion	136

Chapter 7: The effect of eccentric exercise-induced muscle damage on the dynamics of muscle oxygenation and pulmonary oxygen uptake

7.1	Abstract	138
7.2	Introduction	139
7.3	Methods	141
7.4	Results	150
7.5	Discussion	157
7.6	Conclusion	166

Chapter 8: Conclusions

8.1	Main findings	168
	8.1.1 <i>The ventilatory response to dynamic exercise with EIMD</i>	168
	8.1.2 <i>The gas exchange threshold (GET) and EIMD</i>	169
	8.1.3 <i>The perception of exertion during dynamic exercise with EIMD</i>	170
	8.1.4 <i>Time to exhaustion</i>	171
	8.1.5 <i>The metabolic response to dynamic exercise with EIMD</i>	172
	8.1.6 <i>Oxygen uptake kinetics and muscle oxygenation</i>	173
8.2	Limitations	174
8.3	Implications and future directions	175

8.4	Conclusion	176
Chapter 9:	References	178
Chapter 10	Appendices	
Appendix A	Exemplar application for ethical approval	215
Appendix B	Exemplar participant information sheet	222
Appendix C	Exemplar participant consent form	224
Appendix D	Exemplar ethical approval certificate	225
Appendix E	Exemplar risk analysis: squatting protocol	226
Appendix F	Borg (6-20) RPE scale	228
Appendix G	Visual analogue scale (VAS)	230
Appendix H	Statistical assumptions	231
Appendix I	³¹ P-MRS muscle metabolic responses	233
Appendix J	Schematic demonstrating the reduced diffusing capacity of muscle following eccentric muscle damaging exercise	234

List of Figures

- Figure 1.1** Postulated series of events leading to muscle damage from eccentric exercise (Proske & Morgan, 2001, p. 334).
- Figure 1.2** The relationship between length and tension in skeletal muscle. Adapted from Gordon et al. (1966), p.185.
- Figure 1.3** Critical stages in the increase of myofilament overlap corresponding to key points (1–6) labelled on the length-tension curve in figure 2.1. Adapted from Gordon et al. (1966), p.186.
- Figure 2.1** Longitudinal sections of fast-twitch (FT) fibres in A) triceps brachii muscle of a sedentary control rat, and B) rat triceps brachii muscle 1 day after exercise downhill running (DH). Scale bars, 1 μ m. Adapted from Takekura et al. (2001).
- Figure 2.2** Hamstrings angle-torque curves before eccentric exercise (Control) (○) and immediately post exercise (●). Gaussian curves have been fitted to the top 10% of each curve. Adapted from Brockett et al., (2001). Note the immediate right shift of the angle-torque relationship following eccentric exercise
- Figure 3.1** A participant being guided in the correct and safe squatting technique prior to completion of 100 (Smith) squats.
- Figure 3.2** The assessment of isokinetic peak torque, using a Biodex B-2000 isokinetic dynamometer (Biodex Corp, Shirley, NY).
- Figure 4.1** A participant reporting his RPE during cycle exercise using the Borg 6-20 RPE Scale.
- Figure 4.2** Minute values (severe intensity exercise). Minute-by-minute changes in **a:** breathing frequency (f_R), **b:** minute ventilation (\dot{V}_E), **c:** oxygen uptake ($\dot{V}O_2$), **d:** HR, **e:** $\dot{V}_E/\dot{V}O_2$ and **f:** RPE during cycling at 70% Δ pre- and 48h post-eccentric exercise. Values are mean (\pm SEM).
- Figure 4.3** Percentage time values (severe intensity exercise). Changes in **a:** breathing frequency (f_R), **b:** minute ventilation (\dot{V}_E), **c:** oxygen uptake ($\dot{V}O_2$), **d:** HR, **e:** $\dot{V}_E/\dot{V}O_2$ and **f:** RPE during cycling at 70% Δ pre- and 48h post-eccentric exercise set against % time to volitional exhaustion. Values are mean (\pm SEM).
- Figure 5.1** A participant performing a ramp incremental cycle exercise test.

- Figure 5.2** Representative response of $\dot{V}CO_2$ vs $\dot{V}O_2$ (panel A) and blood [lactate] (panel B) showing the region of interest, pre- (●) and 48 h post- (○) eccentric exercise respectively. Best-fit S1 slopes and vertical arrows indicating the gas exchange threshold (GET, panel A) and lactate threshold (Tlac, panel B) illustrate changes in the GET but not the Tlac response, pre- (solid line) and post- (dashed line) eccentric exercise.
- Figure 5.3** Changes in ratings of perceived exertion (RPE) as a function of $\dot{V}O_2$, pre- (●) and 48 h post- (○) eccentric exercise respectively. The vertical arrow indicates the gas exchange threshold (GET) pre-eccentric exercise. There is a 7% increase ($P < 0.05$) in RPE 48 h post eccentric exercise at the $\dot{V}O_2$ value of the pre-eccentric exercise GET.
- Figure 5.4** Percentage changes in minute ventilation ($\dot{V}E$), expired CO_2 ($\dot{V}CO_2$), respiratory exchange ratio (RER), ventilatory equivalent for O_2 ($\dot{V}E/\dot{V}O_2$), ventilatory equivalent for CO_2 ($\dot{V}E/\dot{V}CO_2$), breathing frequency (f_R) and tidal volume (V_T) at the $\dot{V}O_2$ value of the pre-eccentric exercise GET. Values are mean (\pm SD).
- Figure 6.1** The MRS knee extensor ergometer showing pulley system and load basket
- Figure 6.2** [PCr] response of a representative participant, pre- and post-eccentric, muscle damaging exercise. Vertical arrows in indicate time to exhaustion in the two exercise conditions. The solid line represents time to exhaustion pre-eccentric exercise; the dashed line represents time to exhaustion post-eccentric exercise.
- Figure 6.3** [Pi]:[PCr] response of a representative participant, pre- and post-eccentric, muscle damaging exercise. Vertical arrows indicate time to exhaustion in the two exercise conditions. The solid line represents time to exhaustion pre-eccentric exercise; the dashed line represents time to exhaustion post-eccentric exercise.
- Figure 6.4** pH response of a representative participant, pre- and post-eccentric, muscle damaging exercise. Vertical arrows in indicate time to exhaustion in the two exercise conditions. The solid line represents time to exhaustion pre-eccentric exercise; the dashed line represents time to exhaustion post-eccentric exercise.
- Figure 7.1** Schematic overview of experimental procedures. $\dot{V}O_2$ max, Maximal oxygen uptake.
- Figure 7.2** Measurement principle and probe structure of the NIRO 300 (Hamamatsu, Hamamatsu Photonics KK, Japan)

- Figure 7.3** Attachment of the NIRS probes. The NIRS probe holder is secured by means of a double-sided adhesive (a). The thigh with attached NIRS probe holder is wrapped in an elastic bandage (b).
- Figure 7.4** A representative subject's $\dot{V}O_2$ response to severe cycle exercise pre- (●) and 48 h post- (○) eccentric exercise. The vertical line represents the transition from 'unloaded' to 'loaded' cycling.
- Figure 7.5** Deoxygenated Hb ([HHb]) response to severe cycle exercise pre- (●) and 48 h post- (○) eccentric exercise in a representative subject. The vertical line represents the transition from unloaded to loaded cycling. Note the slower overall [HHb] kinetics following eccentric exercise. AU, arbitrary units.
- Figure 7.6** Mean initial [HHb] response to severe cycle exercise pre- (●) and 48 h post- (○) eccentric exercise. The vertical line represents the transition from 'unloaded' to 'loaded' cycling. The data are normalized for amplitude of the response at baseline. Values are mean (\pm SE). AU, arbitrary units.
- Figure 7.7** Mean difference in the initial [HHb] response to severe intensity exercise pre- and 48 h post eccentric exercise (i.e. pre- minus post-eccentric exercise data). AU, arbitrary units.
- Figure 7.8** Total haemoglobin response to severe cycle exercise pre- (●) and 48 h post- (○) eccentric exercise. AU, arbitrary units.

List of Tables

- Table 4.1** Participant characteristics ($N = 10$).
- Table 4.2** Changes in markers of muscle damage. Mean (\pm SD) values before (pre) and 30min and 48h after eccentric exercise
- Table 4.3** Changes in f_R , \dot{V}_E , $\dot{V}O_2$, HR, $\dot{V}_E/\dot{V}O_2$ and RPE during moderate intensity exercise. Mean (\pm SD) values before (pre) and 48h after (post) eccentric exercise.
- Table 5.1** Changes in indicators of muscle damage. Mean \pm SD values before (pre) and at 30 min, 24 h and 48 h after eccentric exercise.
- Table 5.2** Peak values attained during ramp exercise tests. Mean \pm SD values before (pre) and 48 h after (post) eccentric exercise
- Table 5.3** Values attained at the gas exchange threshold (GET) during ramp exercise tests. Mean \pm SD values before (pre) and 48 h after (post) eccentric exercise
- Table 6.1** Changes in indicators of muscle damage. Mean \pm SD values and (range) before and 24 h and 48 h after eccentric exercise.
- Table 6.2** Changes in muscle metabolic responses before and 48 h after eccentric exercise. Values are mean \pm SD.
- Table 7.1** Changes in markers of muscle damage. Mean \pm SD values before (pre) and at 24 h and 48 h after eccentric exercise
- Table 7.2** Pulmonary O_2 uptake responses to severe intensity exercise before and after eccentric muscle damaging exercise
- Table 7.3** Ratings of Perceived Exertion (RPE), Heart Rate (HR) and blood lactate concentration ([La]) during severe intensity exercise pre- and 48 h post-eccentric exercise.
- Table 7.4** [HHb] response to severe intensity constant-load exercise pre- and 48 h post-eccentric exercise.

CHAPTER ONE
INTRODUCTION

1.1 Introduction

Exercise-induced muscle damage (EIMD) is a commonly experienced phenomenon resulting from unaccustomed exercise, particularly exercise with a high eccentric component. Eccentric contractions involve the active lengthening of the muscle and occur in every day activities such as walking downstairs. Many athletic activities also involve eccentric muscle action, particularly during the landing or impact phase of running, jumping or turning. Compared to concentric (shortening) and isometric (static) contractions, eccentric muscle action is mechanically more efficient but employs a unique activation strategy which predisposes the muscle to damage (Enoka, 1996; McHugh et al., 2000).

1.2 Mechanisms of muscle damage

Proske and Morgan (2001) have postulated the initial series of events to explain how muscle damage results from eccentric exercise (Figure 1.1). The model suggests that during eccentric contractions weaker sarcomeres are stretched until they are beyond myofilament overlap. This is understood to occur during the descending limb of the length-tension curve where non-uniformity in sarcomere length is known to develop (see Figures 1.2 & 1.3) (Gordon et al., 1966). This leads to sarcomere disruption which is then followed by membrane damage and subsequent dysfunction of the excitation-contraction (E-C) mechanism. Muscle biopsy data provide evidence of disrupted sarcomeres, including Z-line streaming (Newham et al., 1983a; Fridén, 1984), and of damage to t-tubules, sarcoplasmic reticulum and sarcolemma (Lieber et al., 1994, 1996; Fridén & Lieber, 1998).

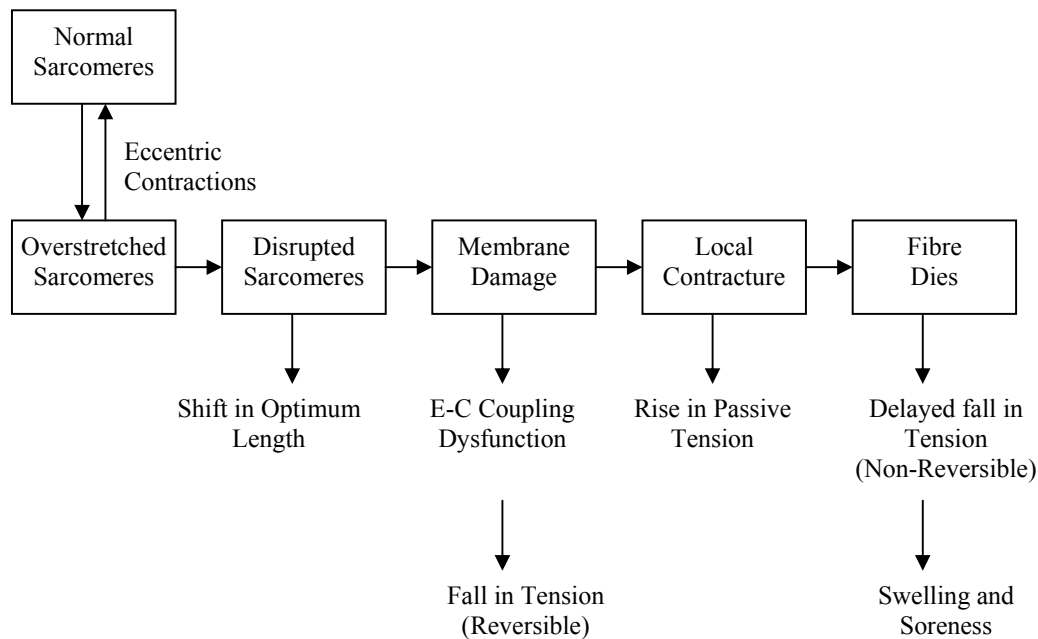


Figure 1.1 Postulated series of events leading to muscle damage from eccentric exercise (Proske & Morgan, 2001, p. 334).

This disruption is reported to occur predominantly in type II fibres, suggesting that they are preferentially damaged (Fridén et al., 1983; Fridén & Lieber, 1992). More recently, intravital microscopy observations have also revealed substantial microvascular dysfunction including an increase in non-red blood cell-flowing capillaries and enlarged capillary diameters (Kano et al., 2005). These ultrastructural disturbances initiate an influx of Ca^{2+} into the sarcoplasm which activates proteolytic pathways involved in muscle fibre break-down and repair (Peake et al., 2005, Tidball, 2005). These events in turn produce symptoms associated with muscle damage, including increased intramuscular proteins in the bloodstream (Hortobágyi & Denahan, 1989), prolonged loss of muscular strength, decreased range of motion and increased muscle soreness (Clarkson et al., 1992; Cleak & Eston, 1992).

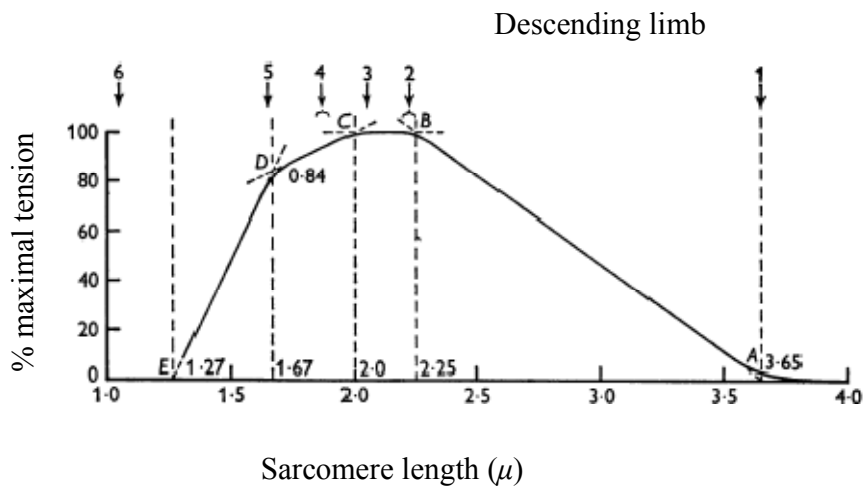


Figure 1.2 The relationship between length and tension in skeletal muscle. Adapted from Gordon et al. (1966), p.185.

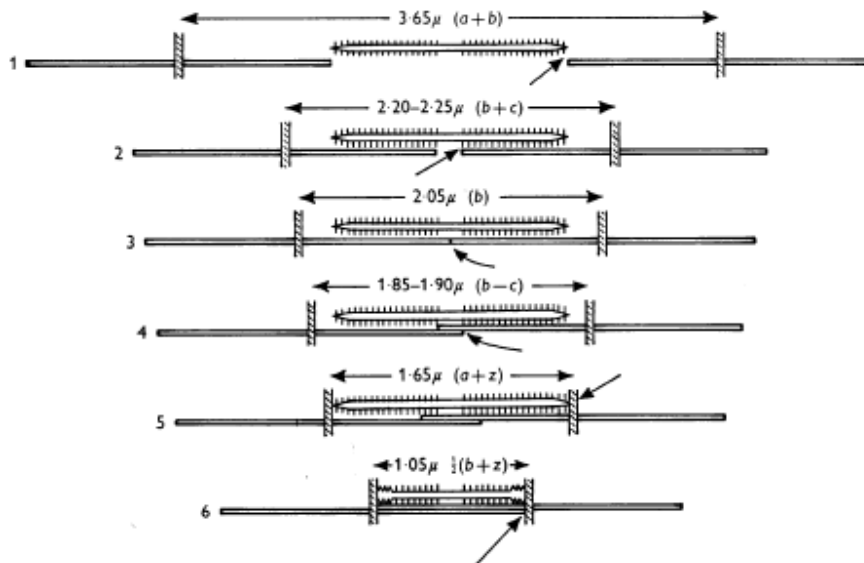


Figure 1.3 Critical stages in the increase of myofibril overlap corresponding to key points (1–6) labelled on the length-tension curve in figure 2.1. Adapted from Gordon et al. (1966), p.186.

1.3 Muscle function following eccentric exercise

Although there are several symptoms associated with EIMD, it has been suggested that measurements of muscle function provide the best means of assessing the magnitude and duration of muscle injury (Warren et al., 1999). Furthermore, the immediate and prolonged loss of force-generating capacity that results from eccentric exercise is possibly the most important symptom when considering the influence of EIMD on the human response to dynamic exercise (Byrne et al., 2004).

Isometric strength measured at a fixed joint angle is the most frequently used assessment of muscle function following eccentric exercise (Warren et al., 1999), with the greatest decrements in maximal voluntary contraction (MVC) occurring immediately after eccentric exercise, followed by a linear recovery (Byrne et al., 2001). Evaluation of dynamic muscle function following EIMD can be achieved using isokinetic dynamometry. Although it is not possible to replicate sport-specific movement patterns and velocities, insights into dynamic muscle function have been gained using this technique. The magnitude of strength loss and rate of recovery between isometric, concentric and eccentric muscle actions appears to be similar following EIMD (Byrne & Eston, 2002a; Michaut et al., 2002). However, conflicting findings have been reported in relation to changes in peak torque at different angular velocities. Observations that decrements in peak torque are greatest at higher angular velocities (Fridén et al., 1983; Eston et al., 1996) support the notion that type II fibres may be selectively damaged during eccentric exercise (Fridén et al., 1983; Fridén & Lieber, 1992); but are countered by conflicting evidence that peak torque is affected to a greater extent at slower rather than faster angular velocities (Gibala et al., 1995; Deschenes et al., 2000; Michaut et al., 2002).

1.4 Dynamic exercise

Athletic performance that relies on the muscle's ability to generate force rapidly is negatively impacted following exercise-induced muscle damage (EIMD). The loss of force-generating capacity results in impaired sprint performances resulting from reductions in peak power and increases in time to reach peak power. Immediate and prolonged reductions have been observed in peak power output on a cycle ergometer (Sargeant & Dolan, 1987; Byrne & Eston, 2002b), in time to peak power (Twist & Eston, 2007) and in cycle sprint performance (Twist & Eston, 2005). However, recovery is not immediate and further decrements at 24 and 48 h are observed, suggesting that delayed onset muscle soreness (DOMS) may influence the dynamic response.

The influence of EIMD on endurance performance remains in dispute. Some studies have reported no change in sub-maximal running performance following a prior bout of eccentric muscle-damaging exercise (Hamill et al., 1991; Scott et al., 2003; Paschalis et al., 2005; Marcora & Bosio, 2007). Whereas other investigations have observed that EIMD has a negative effect on performance (Braun & Dutto, 2003; Chen et al., 2007b). EIMD does not appear to alter the $\dot{V}O_2$ response to sub-maximal cycling (Gleeson et al., 1995, 1998; Walsh et al., 2001; Moysi et al., 2005; Schneider et al., 2007); however the influence of EIMD on other cycling performance measures is uncertain.

1.5 Summary

The intention of this thesis is to investigate the effects of exercise-induced muscle damage (EIMD) on the response to dynamic exercise in human subjects. This topic has been investigated infrequently and findings are equivocal. Therefore, the main objective is to

provide empirical evidence to advance the scientific knowledge and understanding of the phenomenon of EIMD; principally by investigating the physiological, perceived exertion and metabolic responses to the performance of dynamic exercise with EIMD. This thesis comprises 4 studies, as outlined below. Studies 1 and 4 used a total sample of 13 participants, 7 of which were common to both studies. Similarly, studies 2 and 3 used a total sample of 16 participants, 7 of which were common to both studies.

Study 1 The effect of exercise-induced muscle damage on ventilatory and perceived exertion responses to moderate and severe intensity cycle exercise

This study examined the effect of exercise-induced muscle damage (EIMD) on ventilatory and perceived exertion responses to cycle exercise. Ten healthy, physically active men cycled for six minutes at moderate intensity and to exhaustion at severe intensity before and 48 h after eccentric exercise (100 squats with a load corresponding to 70% of body mass). Changes in ventilation and ratings of perceived exertion (RPE) were calculated for each individual and expressed against time (moderate and severe exercise) and as a percentage of time to exhaustion (severe exercise). Ventilation increased during moderate exercise at 48 h (\dot{V}_E ; 34.5 ± 5.0 to 36.3 ± 3.8 L \cdot min $^{-1}$, $P < 0.05$) but increases in RPE were not significant. During severe exercise at 48 h, time to exhaustion (TTE) was reduced and \dot{V}_E (87.1 ± 14.1 to 93.8 ± 11.7 L \cdot min $^{-1}$) and RPE (15.5 ± 1.3 to 16.1 ± 1.4) were elevated ($P < 0.05$). When expressed as a percentage of TTE, the differences in ventilation and RPE values disappeared. Findings indicate that the augmented ventilatory response to cycle exercise following EIMD may be an important cue in informing effort perception during high intensity exercise but not during moderate intensity exercise.

No change was observed in the blood lactate response, although the ventilatory response was enhanced, indicating that other ventilatory stimuli may play an important role following EIMD. The potential dissociation of ventilatory and blood lactate responses during ramp incremental cycling following EIMD was investigated in study 2.

This study has formed the basis of the following publication:

Davies, R. C., Rowlands, A. V., & Eston, R. G. (2009). Effect of exercise-induced muscle damage on ventilatory and perceived exertion responses to moderate and severe intensity cycle exercise. *European Journal of Applied Physiology*, 107(1), 11-19.

Study 2 The effect of eccentric exercise-induced muscle damage on the gas exchange threshold

A prior bout of eccentric muscle-damaging exercise augments the ventilatory response to constant-load cycle exercise without altering the blood lactate profile. However, the influence of exercise-induced muscle damage (EIMD) on the performance of ramp incremental exercise has received sparse attention. This study tested the hypothesis that EIMD would augment the ventilatory response to subsequent ramp incremental cycle exercise leading to a reduction in the gas exchange threshold (GET). In the absence of an altered blood lactate profile this would indicate a dissociation of the GET from the lactate threshold. Ten healthy, physically active male subjects (age, 25 ± 7 years; mass, 80.1 ± 9.9 kg; height, 1.80 ± 0.08 m) performed maximal incremental cycle exercise tests before (pre) and 48 h after (post) completing 100 squats, with a load corresponding to 70% body mass. Following eccentric exercise GET occurred earlier (pre GET $\dot{V}O_2$: 1.58 ± 0.26 ; post GET $\dot{V}O_2$: 1.41 ± 0.14 l.min⁻¹) while the blood lactate response was unchanged ($P > 0.05$). The dissociation between GET and the blood lactate response during cycling with EIMD indicates that the two phenomena are not causally linked. We propose that the 13% increase in ventilation and resultant reduction in GET are evoked predominantly by

increased activation of group III and IV afferents which are stimulated via the mechanical disruption of muscle fibres and local microvasculature due to eccentric exercise.

The decreased endurance capacity observed may have resulted from a shift in the muscle metabolic profile to an increased reliance on non-oxidative metabolism. Therefore, the purpose of study 3 was to investigate alterations in muscle metabolism during incremental exercise to exhaustion.

This study is currently under review for publication:

Davies R.C., Rowlands A.V., Poole D.C., Jones A.M. and Eston R.G. Exercise-induced muscle damage dissociates the Lactate and Gas exchange thresholds. *Currently under review.*

Study 3 The ³¹P-MRS metabolic response to incremental exercise following eccentric, muscle-damaging exercise

Performance decrements associated with EIMD include a reduction in maximal force-generating capacity (Clarkson et al., 1992) and a shorter time to exhaustion (Asp et al., 1998; Carmichael et al., 2005, 2006). Asp et al. (1998) have proposed that EIMD results in a shift to more glycolytic energy production which may contribute to the accelerated development of fatigue. This study investigated the influence of EIMD on changes in muscle metabolism during incremental exercise using ³¹phosphorus magnetic resonance spectroscopy (³¹P-MRS). Before and 48 h after performing 100 squats, ³¹P-MRS was used to measure dynamic changes in [PCr], [Pi], [ADP] and pH during knee-extensor incremental tests to exhaustion. The resting [Pi]:[PCr] ratio was increased 48 h after eccentric exercise (pre: 0.12 ± 0.02; post: 0.18 ± 0.05). During incremental exercise the changes in pH, [PCr] and [Pi]:[PCr] were similar but did not continue for as long. Time to exhaustion and associated peak work rate values were significantly reduced following eccentric exercise (pre: 519 ± 56; post: 459 ± 63 s) and (pre: 29 ± 4; post: 25 ± 4 W),

respectively. End exercise pH and [PCr] values were significantly higher (pre: 6.75 ± 0.12 ; post: 6.83 ± 0.15) and (pre: 20 ± 13 ; post: 33 ± 15 % baseline values), respectively. These findings demonstrated that the accelerated development of fatigue following eccentric exercise did not result from alterations in muscle phosphate metabolism.

While phosphate metabolism does not appear to have a detrimental influence on the performance of dynamic exercise, the substantial microvascular dysfunction observed following EIMD (Kano et al., 2005) may contribute to impaired performance due to disruptions to delivery and distribution of O_2 within the capillary bed of the active muscle. Thus study 4 was designed to investigate the influence of EIMD on the matching of O_2 delivery to O_2 utilisation.

This study is currently under review for publication:

Davies RC, Eston RG, Fulford J, Rowlands AV and Jones AM. Muscle damage alters the metabolic response to dynamic exercise in humans: a ^{31}P -MRS study. *Currently under review*.

Study 4 The effect of eccentric exercise-induced muscle damage on the dynamics of muscle oxygenation and pulmonary oxygen uptake

Unaccustomed eccentric exercise has a profound impact on muscle structure and function. However, it is not known whether associated microvascular dysfunction disrupts the matching of O_2 delivery ($\dot{Q}O_2$) to O_2 utilisation ($\dot{V}O_2$). Near infra-red spectroscopy (NIRS) was used to test the hypothesis that eccentric exercise-induced muscle damage would elevate the muscle $\dot{Q}O_2:\dot{V}O_2$ ratio during severe intensity exercise whilst preserving the speed of the $\dot{V}O_2$ kinetics at exercise onset. Nine physically active men completed 'step' tests to severe-intensity exercise from an unloaded baseline on a cycle

ergometer before and 48 h after eccentric exercise (100 squats with a load corresponding to 70% of body mass). NIRS and breath-by-breath pulmonary $\dot{V}O_2$ were measured continuously during the exercise tests and subsequently modelled using standard non-linear regression techniques. There were no changes in phase II pulmonary $\dot{V}O_2$ kinetics following the onset of exercise (time constant, pre: 25 ± 4 ; post: 24 ± 2 s; amplitude, pre: 2.36 ± 0.23 ; post: 2.37 ± 0.23 L/min; all $P > 0.05$). However, the primary (pre: 14 ± 3 ; post: 19 ± 3 s) and overall (pre: 16 ± 4 ; post: 21 ± 4 s) mean response time of the [HHb] response was significantly slower following eccentric exercise ($P < 0.05$). The slower [HHb] kinetics observed following eccentric exercise is consistent with an increased $\dot{Q}O_2 : \dot{V}O_2$ ratio during transitions to severe-intensity exercise. We propose that unchanged primary phase $\dot{V}O_2$ kinetics are associated with an elevated $\dot{Q}O_2 : \dot{V}O_2$ ratio that preserves blood-myocyte O_2 flux.

This study has formed the basis of the following publications/presentations:

Publication:

Davies, R. C., Eston, R. G., Poole, D. C., Rowlands, A. V., Dimenna, F., Wilkerson, D. P., et al. (2008). The effect of eccentric exercise-induced muscle damage on the dynamics of muscle oxygenation and pulmonary oxygen uptake. *Journal of Applied Physiology*, 105(5), 1387-1388.

Because the reviewers thought the paper could have substantial impact on the field, Dr. Mark Burnley was invited to write a Commentary to accompany the publication of the article:

Burnley M. (2008). Found in translation: the dependence of oxygen uptake kinetics on O_2 delivery and O_2 utilization. *Journal of Applied Physiology*, 105(5), 1387-1388

Presentation

Davies R, Eston R, Poole D, Rowlands A, Dimenna F, Wilkerson D, Twist C, and Jones A. (2008). The influence of exercise-induced muscle damage on pulmonary oxygen uptake and muscle deoxygenation kinetics during high intensity exercise. In J. Cabri, F. Alves, D. Araujo, J. Barreiros and A. Veloso (Eds), *13th Annual Congress of the European Congress of Sports Sciences, Book of Abstracts* (p630). Estoril.

CHAPTER TWO
REVIEW OF THE LITERATURE

2.1 Introduction

The temporary damage to skeletal muscle that results from unaccustomed exercise has been the subject of investigation by exercise physiologists for over 100 years. In 1902 Hough described the delayed but transient soreness experienced when untrained muscle made a series of contractions against a strong spring, suggesting that it resulted from ruptures within the muscle. Since then, several hundred published investigations have attempted to elucidate the underlying mechanisms of this phenomenon and document its symptoms. Direct histological analysis of both human and animal muscle tissue has provided evidence to support Hough's (1902) original contention that changes in skeletal muscle morphology are symptomatic of unaccustomed exercise.

2.2 Changes in skeletal muscle morphology

Human muscle biopsy data has provided direct evidence of considerable disruption in the ultrastructure of skeletal muscle following unaccustomed eccentric exercise (Fridén et al., 1981, 1983; Fridén, 1984; Newham et al., 1983a; Jones et al., 1986; Gibala et al., 1995). Disturbances which originate in the myofibrillar Z-band are seen as streaming, broadening or total disruption. Z-line disruption is the most frequently reported ultrastructural abnormality and as such may represent the weak link in the myofibril contractile mechanism (Newham et al., 1983a; Fridén 1984). Reports of greater disruption of type II fibres have led to the understanding that these fibres are preferentially damaged during eccentric exercise (Fridén et al., 1983; Fridén, 1984; Jones et al., 1986; Lieber et al., 1991; MacPherson et al., 1996). Eccentric contractions are understood to generate higher levels of mechanical stress than concentric or isometric contraction due to reduced motor unit activation (Enoka, 1996; Armstrong et al., 1991; McHugh et al., 2000). This has led to

speculation that type II motor units are selectively recruited during eccentric contraction (Enoka, 1996; McHugh et al., 2000, 2002; Nardone & Schieppati, 1988; Nardone et al., 1989; Howell et al., 1995) and that the excessive stress on the small number of active fibres leads to them becoming damaged (McHugh et al., 2000).

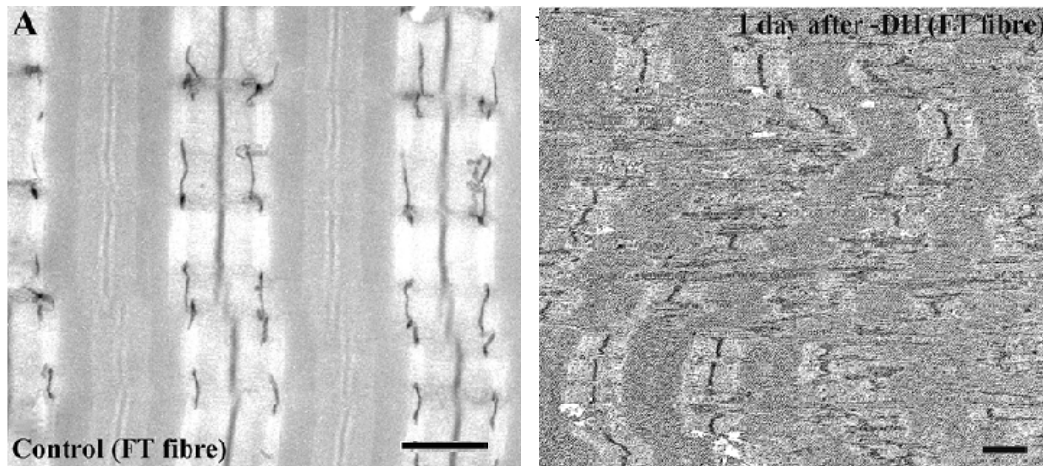


Figure 2.1 Longitudinal sections of fast-twitch (FT) fibres in A) triceps brachii muscle of a sedentary control rat, and B) rat triceps brachii muscle 1 day after exercise downhill running (DH). Scale bars, 1µm. Adapted from Takekura et al. (2001). Note the Z-line smearing and focal disruption of the A-band region following downhill running.

In animal models used to study EIMD, damage to contractile and cytoskeletal components of predominantly type II fibres has been revealed using histological staining (Fridén and Lieber, 1992; Takekura et al., 2001) (Figure 2.1). Lack of staining for cytoskeletal proteins has provided evidence of disruption to the sarcolemma and cytoskeleton (Lieber et al., 1994, 1996; Fridén & Lieber, 1998; Komulainen et al., 1998, 1999, 2000). Morphological changes in skeletal muscle also include ultrastructural changes in the arrangement of the T-tubules which is believed to occur when adjacent myofilaments show disparate degrees of stretch (Takekura et al., 2001; Yueng et al., 2002), and the presence of multiple central

nuclei (Kano et al., 2004). Furthermore, disruption of capillary geometry including enlarged capillary diameters has been observed using intravital microscopy, possibly resultant to myocyte swelling (Kano et al., 2004).

In human studies changes in the intermediate filament system have been interpreted as evidence of myofibrillar and cytoskeletal damage (Fridén et al., 1984). However, this interpretation has been challenged by Yu and colleagues (2002) who have reported increased rather than decreased staining of cytoskeletal proteins. These authors suggested that their findings provide evidence of muscle repair and remodelling rather than muscle damage from eccentric contraction (Yu et al., 2002, Yu & Thornell, 2002). Whilst animal models may not always accurately reflect changes in their human counterparts, they have provided valuable insights which are not so easily procured in human models. Indeed, caution should be taken when interpreting the results of studies using human muscle biopsy as there is evidence to suggest that the biopsy procedure itself may produce some changes mistakenly attributed to EIMD (Malm et al., 2000; Roth et al., 2000). Nonetheless direct analysis of muscle tissue has revealed substantial disruption in both human and animal models of EIMD.

2.3 Indirect markers of muscle damage

Due to the invasive nature of muscle biopsy procedures in human investigations research scientists have increasingly selected to utilise indirect markers of muscle damage in an attempt to further our understanding of the underlying mechanisms of EIMD and its symptoms.

2.3.1 Muscle protein efflux

The structural and functional status of muscle tissue can be determined by the level of skeletal muscle enzymes in the blood. If sarcolemmal integrity is compromised as a result of eccentric exercise (as reported in 2.2), an efflux of muscle proteins into the bloodstream will result (Hortobágyi & Denahan, 1989). Muscle proteins such as creatine kinase (CK) and lactate dehydrogenase (LDH) are commonly used as indirect markers of muscle damage. In fact, Warren et al. (1999) reported that, of human studies reviewed, over 50% used changes in blood levels of myofibre proteins as evidence of EIMD with CK being the most frequently reported. However, the time course of CK activity in the blood appears to be dependant on the damage protocol employed. Following muscle-damaging exercise such as downhill running, weightlifting or plyometric exercise, plasma CK levels peak at 24-48 h (Paul et al., 1989; Eston et al., 1996; Horita et al., 1999; Byrne and Eston, 2002a; Twist & Eston, 2005; Chen et al., 2007b). In contrast, high-force eccentric exercise using isokinetic dynamometers induces a delayed response with CK levels peaking at day 4-5 (Clarkson et al., 1992; Nosaka & Clarkson, 1992, Chen et al., 2003, Zainuddin et al., 2005). Interpretation of the CK response to eccentric exercise is further complicated by high inter-subject variability despite similar decrements in contractile function (Clarkson and Ebbeling, 1988; Hortobágyi and Denahan, 1989). Whilst it is compelling to hypothesise a relationship between the loss of sarcolemmal integrity, increase in plasma CK activity and loss of muscle function, there is no evidence that plasma CK levels accurately reflect the extent of myofibre damage caused (Nosaka & Clarkson, 1992).

2.3.2 Calcium homeostasis

Myofibril damage in animal models of EIMD has been associated with the loss in calcium (Ca^{2+}) homeostasis due to disturbances of the myocyte membrane (Armstrong, 1990). The disruption of sarcoplasmic reticulum (SR) (Byrd, 1992; Fridén & Lieber, 1996) increases membrane permeability and is understood to be responsible for increases in intracellular Ca^{2+} concentration (Armstrong, 1984). Increases in Ca^{2+} may then contribute to the further degradation of the muscle tissue by stimulating the release of calcium-activated neutral proteases such as calpain which have been shown to damage Z-line-associated proteins (Busch et al., 1972; Belcastro, 1993; Belcastro et al., 1998). However, the direct investigation of SR regulation of calcium in human EIMD is extremely limited and has produced conflicting results. Nielson et al. (2005) observed no change in SR function following eccentric, muscle-damaging exercise. In contrast, Enns and colleagues (1999) reported no immediate change in SR Ca^{2+} uptake but a prolonged alteration in SR function in the 2-14 day recovery period. The administration of calcium channel blockers (CCB) in animal models of EIMD has been reported to reduce or prevent the rise in intracellular Ca^{2+} and subsequent injury (Soza et al., 1986; Duan et al., 1990; Duarte et al., 1992; Armstrong et al., 1993). Similarly, in humans, damage to some sarcomeric proteins was attenuated or delayed by the administration of CCBs following eccentric exercise (Beaton et al., 2002). However, the administration of CCBs unexpectedly increased the infiltration of inflammatory cells including neutrophils and macrophages into the muscle tissue, possibly due to the influence of CCBs on vascular and smooth muscle tone (Beaton et al., 2002).

2.3.3 Inflammatory response

Following eccentric, muscle-damaging exercise, inflammatory cells such as neutrophils and macrophages are understood to infiltrate the muscle in order to remove necrotic tissue and initiate the process of muscle repair and regeneration (MacIntyre et al., 1995; Peake et al., 2005; Tidball, 2005). The infiltration of these inflammatory cells has also been implicated in producing secondary cytoskeletal disruptions to eccentrically exercised muscle (Pizza et al., 2001, 2005)

The first inflammatory cells to accumulate are neutrophils (Fielding et al., 1993; Malm et al., 2000). These cells remove necrotic tissue by phagocytosis and release cytokines to attract additional inflammatory cells. Neutrophils can permeate human skeletal muscle within an hour of eccentric exercise and remain present for up to 5 days (Fielding et al., 1993) although significant increases are more commonly reported to last up to 24 h (MacIntyre et al., 1996, 2000, 2001, Beaton et al., 2002). Neutrophil accumulation is understood to activate resident macrophages and attract further macrophage invasion. Macrophages are not only actively phagocytic but may also promote repair and regeneration via the release of cytokines known to cause myoblast proliferation in vitro (Hawke & Garry, 2001). However, a recent review has revealed that only 55% of human studies, as opposed to 85% of animal studies, have detected neutrophil infiltration in exercise-damaged muscle (Schneider & Tiidus, 2007). Animal models of EIMD are most commonly used to study inflammatory cell accumulation due to the difficulties inherent in multiple biopsy procedure in human subjects (Pizza et al., 2008). Thus the inflammatory response to eccentric exercise in humans remains controversial, not least because it appears to be dependent on a wide variety of factors including the mode, intensity and duration of

exercise, the muscle groups examined and the method of detection (directly using muscle biopsy or indirectly via blood analysis) (Peake et al., 2005; Tidball, 2005; Schneider & Tiidus, 2007).

2.3.4 Impaired metabolism

A number of studies have indicated that intramuscular glycogen stores are depleted following eccentric, muscle damaging exercise. O'Reilly et al. (1987) demonstrated a prolonged depletion of muscle glycogen content following 45 min of eccentric cycling. Muscle biopsy samples showed that muscle glycogen content had dropped to 61% of baseline values immediately after eccentric exercise and were further depleted to 44% of baseline values 10 days after the exercise bout. Subsequent studies have corroborated these findings which indicate that EIMD may impair muscle glycogen resynthesis (Asp et al., 1995; Asp et al., 1998; Costill et al., 1990).

Resting muscle glycogen uptake is decreased following eccentric exercise due to a decrease in insulin sensitivity. The transient insulin resistance reported following eccentric exercise (Kirwan et al., 1992; del Aguila et al., 2000; Asp et al., 1996) has been linked to the decrease in a major glucose transport protein, GLUT-4 (Asp et al., 1995; Asp et al., 1996). The translocation of GLUT-4 to the cell membrane when additional glucose is required is initiated by the binding of insulin to membrane-bound insulin receptors (IRS-1) (Tee et al., 2007). The physiological stress associated with EIMD appears to impair the insulin stimulation of IRS-1 and the subsequent activation of GLUT-4, leading to decreased insulin-mediated glucose uptake (del Aguila et al., 2000).

While type I muscle fibres are predominantly oxidative, type II fibres, which are selectively recruited (Enoka, 1996; McHugh et al., 2000, 2002; Nardone & Schieppati, 1988; Nardone et al., 1989; Howell et al., 1995) and preferentially damaged (Fridén et al., 1983; Fridén, 1984; Jones et al., 1986; Lieber et al., 1991; MacPherson et al., 1996) during eccentric contractions, are predominantly glycolytic. Asp et al. (1998) have reported that the resting glycogen content of type II fibres is more severely depleted than that of type I fibres following eccentric exercise (Asp et al., 1998). This observation has led to speculation that increased glycogenolysis may result from EIMD and could also be responsible for the higher resting blood lactate concentration [La] reported with EIMD (Asp et al., 1996, Asp et al., 1998). Elevated [La] has also been reported during exercise with EIMD and has similarly been attributed to a shift to more glycolytic energy production (Braun & Dutton, 2003; Chen et al., 2007b, 2008; Gleeson et al., 1995, 1998). It has been suggested that damage to type II fibres would necessitate a greater recruitment of these fibres during exercise with EIMD in order to maintain the required force production (Gleeson et al., 1998). However, higher [La] does not appear to be an obligatory consequence of EIMD. Several studies have reported no change in the [La] response to exercise following prior eccentric exercise (Hamill et al., 1991; Scott et al., 2003; Marcora & Bosio, 2007; Moysi et al., 2005). A recent study by Schneider et al. (2007) reported unchanged phase II $\dot{V}O_2$ kinetics indicating that EIMD did not compromise oxidative function. These authors suggested that the increase in [La] observed must therefore result from an increased lactate efflux from the active muscle due to increased membrane permeability following muscle-damaging exercise (Schneider et al., 2007).

2.3.5 Delayed onset muscle soreness

Delayed onset muscle soreness (DOMS) was first described by Hough (1902) and is the characteristic manifestation most commonly associated with EIMD. In a review of measurement tools used to assess EIMD, Warren et al. (1999) reported that subjective and objective assessment of DOMS was reported in 63% and 12% of the human studies reviewed, respectively; the most frequently observed measurement of muscle damage employed (Warren et al., 1999). However, a poor temporal relationship exists between DOMS and changes in muscle morphology (Newham et al., 1983a; Jones et al., 1986) and changes in muscle function (Newham et al., 1983b; Howell et al., 1993; Rodenburg et al., 1993; Saxton et al., 1995; Prasartwuth et al., 2005). The ‘delay’ in the experience of soreness appears to vary amongst individuals but peak soreness generally develops between 24 and 48 h after the muscle-damaging exercise (Newham et al., 1983b, 1988; Jones et al., 1987, 1989; Clarkson et al., 1992; Cleak & Eston, 1992) and gradually subsides, usually disappearing within 96 h (Jones et al., 1987; Cleak & Eston 1992). In contrast, changes in muscle morphology were found immediately after completion of a 20 min step-test with the severity of the damage increasing in the biopsy samples taken at 24 and 48 hours (Newham et al., 1983a). In an earlier investigation Fridén and colleagues (1981) demonstrated that ultrastructural disturbances were 3 times greater in biopsy samples taken 2 as opposed to 7 days after the eccentric exercise bout although no biopsy was taken immediately after the damage protocol which involved repeatedly running downstairs (Fridén et al., 1981). Functional impairments, in particular changes in strength also follow a different time course to the development of DOMS (Rodenburg et al., 1993; Newham et al., 1983b; Nosaka et al., 2002). Strength decrements are greatest immediately after eccentric exercise and demonstrate a linear recovery back to baseline measurements (Clarkson et al., 1992;

Sayers & Clarkson, 2001; Howell et al., 1993; Byrne & Eston, 2002a) usually within 5-7 days (Armstrong, 1984; Clarkson & Tremblay 1988) although in some individuals, recovery may take up to several weeks (Clarkson et al., 1992; Howell et al., 1993). Thus DOMS is a poor indicator of both functional impairment and the magnitude of morphological muscle damage.

Several theories have been proposed to explain the mechanisms responsible for the sensation of DOMS, including the muscle damage theory originally presented by Hough over 100 years ago (Hough, 1902). The mechanical disruption of structural elements, (for further detail see section 2.2) is believed to contribute to the stimulation of pain receptors (nociceptors). These are primarily group III and IV thin-fibre afferent neurons, located in the muscle, connective tissue, musculotendinous junction, arterioles and capillaries and their stimulation is understood to lead to the sensation of pain (Cheung et al., 2003). Several studies have demonstrated a relationship between increases in muscle soreness and impairments in dynamic muscle function (Horita et al., 1999; Proske et al., 2003; Weerakkody et al., 2003b). However, as explained above, differences in the temporal relationship between DOMS and mechanical disruption indicate that the muscle damage theory can only partially explain the development of DOMS. The noxious stimulus of lactic acid has been implicated in producing the sensation of DOMS (Armstrong, 1984). Elevations in blood lactate concentration [La] have been reported in several studies during exercise following EIMD (Gleeson et al., 1995, 1998; Braun & Dutto, 2003). However, the lactic acid theory has largely been rejected as there appears to be no relationship between ratings of soreness and blood [La] levels following a bout of downhill running designed to

induce EIMD (Schwane et al., 1983) and higher levels of lactate have been shown not to induce soreness in concentric exercise (Armstrong, 1984; Schwane et al., 1983).

Inflammation and swelling triggered by the damage is presently the most widely accepted proposed mechanism for DOMS (Smith, 1991, Proske & Morgan, 2001; Cheung et al., 2003). The pain or soreness resulting from eccentric exercise manifests itself as a dull aching pain that is stimulated by either palpation or contraction and is not present at rest (Cleak & Eston, 1992; Avela et al., 1999; Komi, 2000). Several studies have proposed that swelling subsequent to EIMD may be mechanistically implicated in the development of DOMS due to increases in local tissue pressure (Howell et al., 1985; Bobbert et al., 1986; Fridén et al., 1986). Smith (1991) has hypothesised that increases in intramuscular pressure during contraction or palpation would provide sufficient mechanical stimulus for the sensitisation of mechanical nociceptors (Smith, 1991). These thin-fibre afferents are also known to be sensitised by various inflammatory mediators including bradykinins, prostaglandins and histamines which are released during the process of proteolytic breakdown and repair (Clarkson & Hubal, 2002). It has been suggested that the delay in the inflammatory response may account for the delay in the symptoms of DOMS (Smith, 1991). However, Taguchi and colleagues (2005) have demonstrated that chemical stimuli including pH 5.5, adenosine triphosphate, and bradykinin, do not influence the response of group III and IV muscle afferents to eccentric exercise (Taguchi et al., 2005).

2.3.6 *Changes in strength*

The immediate and prolonged loss of strength that occurs after eccentric exercise is one of the most frequently used markers of EIMD. In a review of measurement tools used in

studies of EIMD, Warren et al. (1999) reported that 50% of human studies measured maximal voluntary contraction (MVC) torque, a measure which the authors suggest provides the most accurate and reliable indirect marker of muscle damage in human studies (Warren et al., 1999). An immediate loss of force generating capacity may also be observed following non-damaging concentric exercise but recovery to baseline strength occurs within hours (Newham et al., 1983b; Jones et al., 1989). The greatest strength loss and longest recovery times have been associated with high-force eccentric protocols such as that employed by Newham and colleagues (1987) which involved maximal eccentric contraction of the elbow flexors. These authors and others have reported strength decrements of over 50% compared to pre-exercise values, with a linear recovery to baseline typically lasting 1 - 2 weeks (Newham et al., 1987; Saxton et al., 1995; Nosaka et al., 1991) although in some individuals, recovery may take up to several weeks (Clarkson et al., 1992; Howell et al., 1993). In contrast, downhill running (Eston et al., 1996, Eston et al., 2000) and bar-bell squatting protocols (Byrne & Eston 2002a, 2002b; Moysi et al., 2005) designed to provoke muscle damage, typically generate a more modest loss in force-generating capacity with strength decrements of between 10 and 30% and recovery to baseline occurring within 4-7 days.

2.4 Muscle function following exercise-induced muscle damage

Morgan and Allen (1999) proposed that the earliest events in eccentric, muscle-damaging exercise involve the over-stretching of randomly distributed sarcomeres, although the initial decline in strength may be attributed to metabolic fatigue, damaged muscle or a combination of both (Morgan & Allen 1999). If sarcomeres are stretched to a point of minimal overlap between actin and myosin filaments, cross-bridge formation would be

reduced and the ability to generate force would be compromised (Clarkson et al., 1992). In support of this hypothesis, it has been reported that loss in strength is greater when the damaging exercise is performed with muscle at longer rather than shorter lengths (Newham et al., 1988; Child et al., 1998).

2.4.1 Changes in optimal muscle length

A shift in the length-tension relationship for a given force to longer muscle length following eccentric exercise was first observed by Katz (1939), who proposed that rapid over-stretching of muscle beyond its optimum length was likely to damage the contractile elements. Katz's original (1939) findings based on frog and tortoise muscle have been confirmed by more recent investigations using human models (Jones et al., 1997; Whitehead et al., 1998; Brockett et al., 2001; Philippou et al., 2003) (Figure 2.2).

These findings provide evidence to support the thesis that a longer muscle length is needed to achieve the same myofilament overlap after eccentric exercise due to an increase in series compliance resulting from the over-stretching of sarcomeres (Morgan & Allen, 1999; Proske & Morgan, 2001). In further support of this theory, a greater loss in strength is reported at short as opposed to longer or optimal muscle length following eccentric exercise indicating a shift towards longer muscle lengths for maximal force generation (Saxton & Donnelly, 1996; Child et al., 1998; Byrne et al., 2001; Sayers & Clarkson, 2001; Byrne & Eston, 2002b).

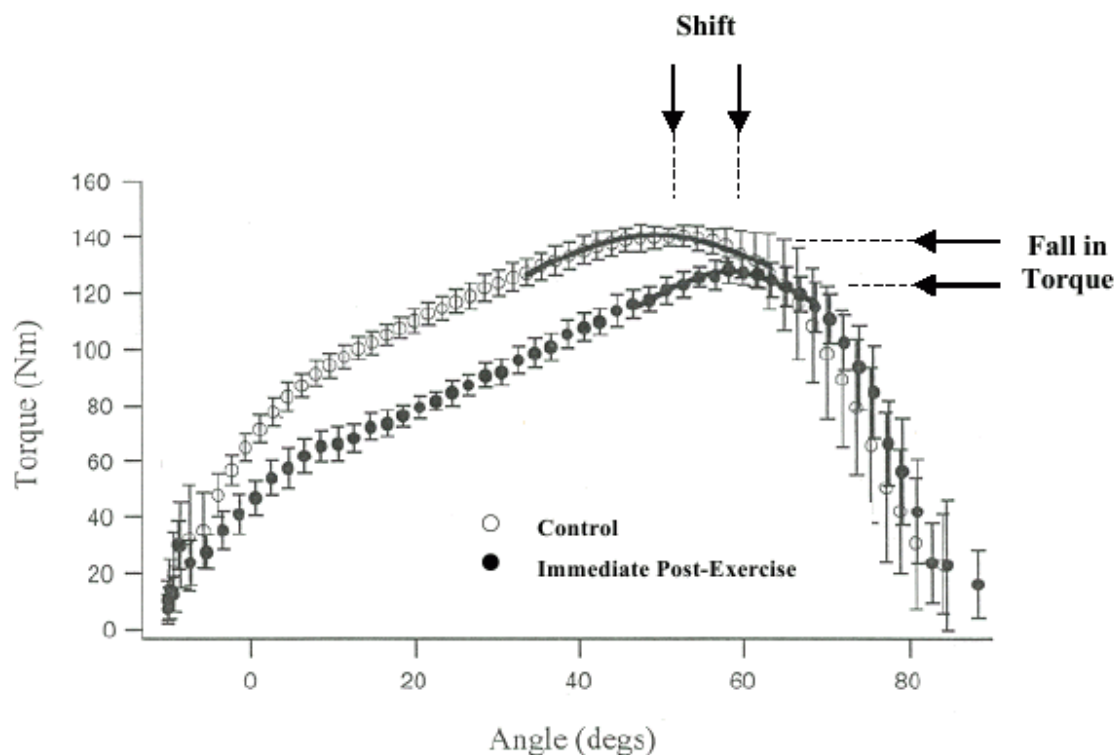


Figure 2.2 Hamstrings angle-torque curves before eccentric exercise (Control) (○) and immediately post exercise (●). Gaussian curves have been fitted to the top 10% of each curve. Adapted from Brockett et al., (2001). Note the immediate right shift of the angle-torque relationship following eccentric exercise.

2.4.2 Low-frequency fatigue

An alternative hypothesis to explain the loss in force-generating capacity and the shift in the length-tension relationship proposes that alterations in calcium homeostasis may be responsible. A decrease in the release of calcium ions (Ca^{2+}) from the sarcoplasmic reticulum (Warren et al., 1993; Westerblad et al., 1993) and an increase in intracellular Ca^{2+} (Chin & Allen, 1996) has been shown to lead to failure of the excitation-contraction (E-C) coupling process in mouse muscle preparations. In human biopsy samples Hill et al. (2001) have reported that sarcoplasmic reticulum Ca^{2+} release and uptake were significantly depressed following exercise that provoked decrements in knee extensor MVC. These and

other authors have also observed a significant correlation between decreases in torque production at low electrical stimulation frequencies and Ca^{2+} release following exercise (Hill et al., 2001, Neilsen et al., 2005).

The disproportionate loss of force at low (20Hz) compared with high (100Hz) frequencies of electrical stimulation is known as low-frequency fatigue (LFF) and is likely caused by an impairment of excitation-contraction coupling process (Edwards et al., 1977). It is known to be induced by fatiguing exercise (Edwards et al., 1977; Newham et al., 1983b; Jones et al., 1989), with the most profound effect provoked by eccentric exercise (Newham et al., 1983b; Jones et al., 1989). Thus there is evidence from both animal and human studies to suggest that reductions in sarcoplasmic reticulum Ca^{2+} release are the primary cause for LFF. However, there is evidence that, in addition to reduced Ca^{2+} release, LFF that follows eccentric exercise may be caused by changes in muscle morphology and subsequent remodelling (Jones et al., 1996; Westerblad et al., 2000). The redistribution of sarcomere lengths following eccentric exercise may account for the length-dependent effect of strength loss associated with EIMD, particularly in view of the fact that, like decrements in MVC, LFF is more evident at short rather than long muscle lengths. (Jones et al., 1989; Byrne et al., 2001)

2.4.3 Alterations to neural control

The generation of muscular force is not only a product of muscle contractile function and E-C coupling but also of neural drive. Therefore the inability to generate maximal force in muscles damaged by eccentric exercise could theoretically be the result of alterations in neural drive, estimates of which can be obtained using electromyography (EMG).

Deschenes et al. (2000) have provided evidence to suggest that neuromuscular efficiency, the ratio of torque generated to integrated EMG (iEMG) activity, is decreased following EIMD. These authors reported increases in iEMG activity and a decrease in the torque:iEMG ratio during maximal isometric contractions which persisted for 10 days. Other EIMD symptoms including plasma CK activity, perceived soreness and peak torque were all recovered within 7 days (Deschenes et al., 2000). Subsequent investigations have reported similar disturbances in the torque:iEMG ratio with the largest effect apparent at low forces; arguably where the precision of force production is most functionally relevant (Weerakkody et al., 2003a; Lavender & Nosaka, 2006; Semmler et al., 2007). Increased isometric force fluctuations have also been observed following eccentric, muscle-damaging exercise but they appear not to be an artefact of muscle damage as measures return to baseline levels within 24 h (Lavender & Nosaka, 2006; Semmler et al., 2007).

Alterations have also been reported in the perception of force production and joint position following eccentric exercise. Saxton et al. (1995) demonstrated that following eccentric exercise of the forearm flexors, participants consistently overestimated the amount of force they could produce. Rather than matching the target force of 35% MVC generated in the undamaged (control) arm, participants undershot their target force for the 5 days of the study following the initial insult (Saxton et al., 1995). However, when the forces applied by both damaged and control arm were expressed as a proportion of the MVC for that arm at that time, errors in estimation were reduced or absent (Saxton et al., 1995). In addition to disturbances in the matching of a sense of force, disturbances in the reproduction of limb or joint position have been reported following eccentric exercise (Saxton et al., 1995; Brockett et al., 1997; Walsh et al., 2004). However, conflicting results were recorded. Brockett et

al. (1997) reported that participants produced larger joint angles following eccentric exercise whereas Saxton et al. (1995) and Walsh et al. (2004) reported that participants produced smaller joint angles following eccentric exercise. It has been proposed that alterations to the perception of force and limb position following eccentric exercise may be due to damaged sensory receptors within the muscle (Saxton et al., 1995; Brockett et al., 1997; Carson et al., 2002; Proske et al., 2003). It is believed the sense of force is derived from peripheral receptors in the muscle and the Golgi tendon organs and that muscle spindles provide the signals for position sense (McCloskey, 1978; Gandevia, 1996). Twist et al. (2008) reported impaired unilateral balance performance 24 h after plyometric, muscle damaging exercise which the authors attributed to alterations in proprioceptive control. However recent work by Gregory and colleagues using anaesthetised cat models has demonstrated that the responsiveness of the tendon organs and muscle spindles is not altered by eccentric exercise (Gregory et al., 2002, 2004). Thus the effect of eccentric, muscle damaging exercise on proprioceptive function is yet to be explained.

It has been proposed that the increases in iEMG associated with altered proprioception following EIMD may be indicative of altered motor unit activation (Proske et al., 2004; Weerakkody et al., 2003b; Prasartwuth et al., 2005; Semmler et al., 2007). Proske et al. (2003) have suggested that DOMS may be involved in alterations to neural control following eccentric exercise. They propose that the pain from DOMS leads to a reduced motor cortical excitability, which may serve to protect muscle during from further damage during the repair process (Proske et al., 2003). However, the mechanism or mechanisms by which EIMD influences alterations in motor unit recruitment remains to be determined.

2.5 The Repeated Bout Effect

The protective adaptation to a single bout of eccentric exercise has been termed the 'repeated bout effect' (Nosaka & Clarkson, 1995). Whenever unaccustomed eccentric exercise is repeated, within a given time-frame, the magnitude of the characteristic symptoms of EIMD is diminished. Changes in muscle morphology, muscle protein efflux, inflammation, loss of strength and other symptoms are attenuated by a repetition of the same exercise but the damaging effects are not prevented (Nosaka & Clarkson, 1995; McHugh et al., 1999; McHugh, 2003). The repeated bout effect may be conferred as early as 2 days after the initial exercise bout (Paddon-Jones et al., 2000; Nosaka & Newton, 2002) and for most of the symptoms of EIMD, lasts at least 6 months but is lost between 9 and 12 months (Nosaka et al., 2001a). Very little prior exercise is needed to confer the effect. As few as 2 maximal eccentric contractions have been demonstrated to confer protection against symptoms of EIMD when the same elbow flexor muscles performed 24 maximal contractions 2 weeks later (Nosaka et al., 2001b). Similarly Lavender and Nosaka (2008) showed that light eccentric exercise, 30 (6 x 5) contractions of just 10% MVC was effective in attenuating muscle damage against a subsequent bout of eccentric exercise of 40% MVC performed 48 h later.

The mechanisms underlying the repeated bout effect are not fully understood although several potential mechanisms have been proposed. It has been suggested that protection is conferred as a result of neural, cellular and mechanical adaptations which may work independently of each other or in concert (McHugh et al., 1999; McHugh 2003). The neural adaptation theory proposes that during a subsequent bout of eccentric exercise, motor unit recruitment is altered in order to redistribute the workload and thus improve

motor unit efficiency (Nosaka & Clarkson, 1995). Warren et al. (2000) observed a 30% decrease in EMG mean frequency in tibialis anterior muscle in the second of two bouts of 50 eccentric MVCs separated by one week. These authors concluded that the data indicated that an increased activation of slow motor units and a concomitant decrease in activation of fast units occurred in the repeated bout (Warren et al., 2000). The findings of Chen (2003) lend further support to the thesis of reduced activation of fast-twitch motor units during the second eccentric bout. However, McHugh et al. (2001) were unable to find any evidence of neural adaptation after performing a repeated bout of relatively low-intensity eccentric exercise.

The cellular adaptation theory involves potential adaptations of the contractile machinery. Proske and Morgan (2001) have suggested that following eccentric exercise optimum muscle length increases due to the addition of sarcomeres in series. A shift of optimum angle towards a longer muscle length following EIMD has been confirmed in several studies (see section 2.4.3). However, in a recent study, Chen and colleagues (2007a) revealed that while a rightward shift in optimum angle demonstrated a relationship with the degree of muscle damage associated with the initial bout, it did not appear to be directly related to the mechanisms responsible for the repeated bout effect (Chen et al., 2007a). Several investigations have used blood markers to show that there is a reduction in the inflammatory response associated with EIMD following a repeated bout of eccentric exercise (Pizza et al., 1996, 2001; Hirose et al., 2004; Smith et al., 2007). However, a more recent study using muscle biopsy, demonstrated that several inflammatory genes were transcriptionally up-regulated (rather than attenuated) following a repeated bout of eccentric leg exercise (Hubal et al., 2008).

Mechanical adaptation to eccentric exercise may involve the remodelling of the intermediate filament system to provide mechanical reinforcement against subsequent bouts. Yu and Thornell (2002) showed that a single bout of downstairs running increased staining of actin and desmin, suggesting that this reflected an increased synthesis of the proteins as part of an adaptation process. More recently Lehti and colleagues (2007) have used rat biopsy data to demonstrate that prior eccentric exercise produced an adaptive response that protected the sarcolemma, intermediate filament, and sarcomeric proteins against subsequent disruption. While there may be several mechanisms underlying the repeated bout effect which may work in isolation or to compliment each other, a unified theory to explain the mechanism of protective adaptation remains elusive.

The recently revealed presence of cross-over or contralateral adaptation to eccentric exercise has indicated that the repeated bout effect is likely to involve a complex interplay of all three proposed mechanisms (Howatson & van Someren, 2007). Following the second bout of 45 eccentric MVCs of the elbow flexors, symptoms of EIMD were diminished when the second bout was performed with the opposite or contralateral limb although the magnitude of change was not as profound as when the same arm was exercised twice. The authors have suggested that the contralateral adaptation was most likely mediated by neural mechanisms as there was no direct stimulus for cellular or mechanical changes to the contralateral arm (Howatson & van Someren, 2007). Thus the more profound adaptation to the same-side arm most likely results from neural, cellular and mechanical mechanisms working in concert.

2.6 Dynamic muscle function

Of all the symptoms of EIMD, the immediate and prolonged impairment to muscle function has the potential to be the most debilitating when considering the human response to dynamic exercise following eccentric exercise. However, the study of dynamic muscle function during athletic performance has received only limited attention. The first study to directly assess the influence of eccentric, muscle-damaging exercise on dynamic muscle function was undertaken by Sargeant and Dolan (1987). These authors employed an exhaustive downhill (-25%) walking intervention to induce damage followed by assessments of maximal short term power output using an isokinetic cycle ergometer. Cycling at 110 rev.min⁻¹ for 20 s, peak power was reduced by 23% 24 h after the eccentric exercise protocol and was still 8% lower than baseline values at 96 h (Sargeant & Dolan, 1987). Decrements in MVC were of a greater magnitude than peak power, with a loss of 45% of pre eccentric exercise values at 24 h, recovering to a 30% loss at 72h (Sargeant & Dolan, 1987).

2.6.1 Wingate 30 s cycle test

These findings have been supported by Byrne and Eston (2002b) using a 30 s Wingate test to investigate changes in power-generating ability following EIMD. These authors reported decrements in peak power output and isometric MVC following the performance of 100 bar-bell squats with a load corresponding to 80% concentric one repetition maximum. There were notable differences in both the magnitude of strength and power loss and their recovery patterns. Isometric MVC dropped by 35% immediately after the squatting protocol and then followed a linear recovery; 26% at 24 h and 19% at 48 h. In comparison, Wingate peak power was reduced by 13% immediately after the eccentric intervention but

dropped further, by 18% at 24 h and 16% at 48 before recovery (Byrne & Eston, 2002b). In a more recent study, Nottle and Nosaka (2007) employed a 40 min downhill (-7%) running damage protocol to investigate changes in Wingate peak power. In agreement with both Sargeant and Dolan (1987) and Byrne and Eston, 2002b), greater decrements were observed in strength than peak power immediately after downhill running (17% and 5%, strength and peak power, respectively), yet neither strength nor power loss persisted beyond this initial post-eccentric exercise measurement. In contrast with the two previous studies (Sargeant & Dolan, 1987; Byrne & Eston, 2002b), recovery was rapid and peak power was unexpectedly 5% higher than baseline measures at 120 h (Nottle & Nosaka, 2007). An earlier investigation, Malm et al. (1999) reported no change in Wingate peak power following a stepping protocol designed to induce muscle damage. However, the modest increase in soreness reported by participants and the unchanged CK response indicate that the stepping protocol employed may not have been sufficiently intense to alter muscle function.

2.6.2 Sprint performance

Malm and colleagues (1999) also reported the changes in the performance of intermittent cycle sprint tests (10 x 10 s all-out cycling interspersed with 50 s rest periods) which unexpectedly improved by 8% at 48 h. No change in 30 m running sprint performance was observed 48 h after the completion of 70 (7 x 10) drop jumps designed to induce muscle damage (Semark et al., 1999). However, participants in the study were well-trained rugby union and field hockey players who, although they reported moderate soreness, showed no elevation in plasma CK levels and may have been protected from alterations to muscle function from the drop jumps via the repeated bout effect (see section 2.5). Effective

muscle-damaging protocols such as the 100 plyometric jumps employed by Highton et al. (2009) have been shown to result in decrements in isokinetic peak torque and increases in 5 and 10 m sprint running times at 24 h and 48 h. Twist and Eston (2005) investigated the influence of EIMD on both cycle and running sprint performance using 100 (10 x 10) counter-movement jumps to induce damage. Peak power output during intermittent cycle sprinting (10 x 6 s with 24 s recovery) was reduced immediately and up to 72 h after eccentric exercise. The rate of fatigue in the cycle tests was also reduced with the greatest decrease observed at 48 h. Similarly intermittent sprint running time (10 x 10 m with 12 s active recovery) increased immediately and up to 48 h after eccentric exercise (Twist & Eston, 2005). In a more recent investigation the same authors compared the performance of a 10 s cycle sprint and a 50 cm drop jump at 24, 48 and 72 h after completing 100 (10 x 10) counter-movement jumps (Twist & Eston, 2007). While performances of both the cycle sprint and the drop jump were reduced as a result of the eccentric exercise the temporal pattern of recovery was different. Peak power output and time to reach peak power were most severely reduced at 48 h, whereas the greatest decrement in drop jump height was observed at 24 h (Twist & Eston, 2007). This observation led the authors to propose that differences in the response to cycling and drop jump performance indicate that the time course of recovery from EIMD is dependent upon the mode of dynamic exercise (Twist & Eston, 2007).

2.6.3 Vertical jump tests

Other studies have compared performance decrements in various vertical jumps in order to investigate the effect of muscle-damaging exercise on dynamic muscle function. Byrne and Eston (2002a) used 100 (10 x 10) barbell squats with a load of 70% body mass to identify

differences in the performance of squat jumps, counter-movement jumps and drop jumps following eccentric, muscle-damaging exercise. The squat jump was performed from a squatting position before jumping vertically for maximal height whereas in both the counter-movement and drop jumps the muscle employed the stretch-shortening cycle (SSC). The SSC of human muscle function is the natural mode of locomotion employed in running, walking or jumping involving the cyclical performance of a sequence of pre-activation, active (eccentric) braking followed by concentric action (Komi, 1984). The reductions in vertical jump performance was immediate and lasted up to 72 h, although squat jump performance was affected to a greater extent than either counter-movement or drop jump performance. This observation prompted the authors to propose that impairment of muscle function in the vertical jumps was attenuated when the SSC was used (Byrne & Eston 2002a). Similar findings have been reported by Harrison and Gaffney (2004) who used 70 (7 x 10) maximal eccentric contractions of the knee extensors to induce muscle damage. In contrast, when muscle damage is induced via a SSC protocol (by means of a specially designed sledge apparatus) rather than a predominantly eccentric protocol, drop jump performance is more profoundly affected than squat jump performance (Avela et al., 1999; Horita et al., 1999, 2003). These authors and others (e.g. Horita et al., 1996, Nicol et al., 2006) have also reported a 'bi-modal' recovery from exhaustive SSC. The bi-modal response involves an immediate post-exercise reduction in dynamic muscle function which is believed to result primarily from metabolic fatigue. This is followed by a partial recovery one to two hours post-exercise and a further reduction in dynamic muscle function which has been attributed to the inflammatory response to EIMD (Nicol et al., 2006). However, the phenomenon of a bimodal or biphasic response may not be exclusive to damage induced via the SSC. MacIntyre and colleagues (1996) were the first to report a biphasic

recovery of dynamic human muscle function following 300 repetitive eccentric contractions of the knee extensors. Furthermore, few studies report measures of muscle function between 0 and 24 h post exercise and those that do more frequently report static force than dynamic torque (Clarkson & Hubal, 2002).

2.6.4 Endurance exercise

The immediate and prolonged loss of power-generating ability that results from muscle damaging exercise also impedes performance during endurance exercise. Mice have been used to investigate running time to exhaustion and the more ecologically valid, voluntary wheel running time, following 150 min downhill running (Carmichael et al., 2005, 2006; Davis et al., 2007). This group of authors has reported decreases in treadmill running time to fatigue at 24, 48 and 72h. When compared to uphill running mice, one downhill group ran 73% less at 24 h and 69% less at 48 h (Carmichael et al., 2005). Voluntary wheel running activity was monitored during the 12 h active dark cycles following downhill running in all three studies. The mice spent shorter time periods on the running wheel and travelled reduced distances in the first and second 12 h active cycles, gradually returning to baseline activity between day 3 (Carmichael et al., 2006; Davis et al., 2007) and day 5 (Carmichael et al., 2005).

To date, only two ecologically valid studies have investigated human endurance performance following EIMD. Marcora and Bosio (2007) reported significant differences in self-paced 30 min time trial running performance before and 48 h after the completion of 100 (10 x 10) drop jumps. Participants ran a shorter distance at a slower speed following EIMD. An analysis of speed, heart rate and perceived exertion variables recorded during

the run indicated that pacing strategy was not altered. In light of these findings the authors proposed that the negative effect of EIMD on time-trial performance was mediated by the individuals' perception of exertion (see section 2.8.2) (Marcora & Bosio, 2007). Twist and Eston (2009) reported similar decrements in 5 min cycle time-trial performance following 100 (10 x 10) countermovement jumps. There were significant reductions in peak power output (-14%), mean power output (-11%), mean revolutions per minute (RPM) (-4%), and distance covered (-4%) 48 h after EIMD but performance measures had returned to baseline at 168 h (Twist & Eston, 2009).

2.7 Human response to dynamic exercise

Investigations reporting changes in physiological parameters during exercise following EIMD have produced equivocal findings. The first study to document physiological changes during exercise with EIMD was undertaken by Hamill and colleagues (1991). Steady-state oxygen uptake ($\dot{V}O_2$) and heart rate (HR) data were recorded during a 15 min run at 80% maximal $\dot{V}O_2$ ($\dot{V}O_{2_{max}}$) before and after a 30 min bout of downhill running (at a gradient of -15%). The $\dot{V}O_2$ and HR responses of the ten recreational runners were unchanged at 48 and 120 h after downhill running. The small, albeit significant increase in CK activity and the development of only moderate soreness led the authors to suggest that the downhill run may have provided insufficient stress to elicit metabolic or HR modifications (Hamill et al., 1991). These authors also reported small but significant decreases in hip and knee flexion during running following EIMD but overall performance of the stride, including stride length, stride period, mechanical work, and mechanical power was unaffected (Hamill et al., 1991).

2.7.1 Oxygen uptake

Measures of $\dot{V}O_2$ during running at a fixed sub-maximal speed, such as those procured by Hamill and colleagues (1991), provide information relating to the cost or economy of locomotion, more commonly termed 'running economy'. In contrast to Hamill et al.'s (1991) findings, Braun and Dutto (2003) reported that running economy was significantly compromised (average 3.2% increase in $\dot{V}O_2$) during 5 min running bouts at 65, 75 and 85% $\dot{V}O_2$ peak, 48 h after a 30 min downhill run (-10%) at an intensity equivalent to 70% $\dot{V}O_2$ peak. Furthermore, these authors reported decreases in the mean stride length at all three exercise intensities which were negatively correlated to changes in the mean energy cost of running at all three exercise intensities (Braun & Dutto, 2003). A comparison of the training status of the two participant groups (Hamill et al., 1991; Braun & Dutto, 2003) was suggested as an important factor in explaining the disparate findings (Braun & Dutto, 2003). The well-trained runners in the Braun and Dutto (2003) study may have had more refined gait patterns than the recreational runners in the Hamill et al. (1991) study. Thus the trained runners may have been more sensitive to changes in gait resulting from muscle damage. In support of this suggestion, Paschalis et al. (2005) reported no change in the economy data of untrained athletes during running at approximately 55 and 75% $\dot{V}O_2$ max during the 3 days following completion of 120 (12 x 10) eccentric MVCs. Similarly, Marcora and Bosio (2007) reported no change in the running economy of active, but not highly trained distance runners, exercising at 70% $\dot{V}O_{2max}$ for 10 min 48 h after the completion of 100 (10 x 10) drops jumps. Another study using active but not highly trained participants also reported unaltered running economy following a series of sub-maximal resistance exercises including barbell squat, weighted lunges and weighted step-ups (Scott

et al., 2003). It was suggested by these authors that the extent of muscle damage induced was insufficient to produce mechanical or physiological changes that would influence $\dot{V}O_2$ (Scott et al., 2003). Chen and colleagues (2007b) have provided the only evidence of the time course of changes in running economy following EIMD. Running economy data were collected for five consecutive days following the completion of a 30 min downhill run (-15%) at an intensity equivalent to 70% $\dot{V}O_2$ peak (Chen et al., 2007b). Using the same submaximal exercise intensities as Braun and Dutto (2003) (65, 75 and 85% $\dot{V}O_2$ peak) these authors demonstrated that running economy was compromised by 4-7% for three days after the downhill running, recovering to near baseline levels on day four (Chen et al., 2007b). In addition, reductions in stride length (3-6%), range of motion (ROM) of the ankle and knee joints (1-7%) and increases in stride frequency (3-7 %) were observed for two to three days following the downhill running. These authors concluded that the time course and magnitude of alterations in economy were more closely related to changes in kinematic parameters than changes in muscle function as indicated by immediate and prolonged changes in MVC (reduction of 7-21% for 4 days after downhill running) (Chen et al., 2007b).

Measures of economy have also been assessed during cycling at a fixed sub-maximal load and are not as susceptible to the effects of alterations in lower limb kinematics as it has been suggested that running economy is. In fact there is consistent evidence that EIMD does not alter cycling economy irrespective of the mode of damage employed or the training status of the participants (Gleeson et al., 1995; Walsh et al., 2001; Moysi et al., 2005; Schneider et al., 2007; Twist & Eston, 2009). Gleeson et al. (1995) and Schneider et al. (2007) employed untrained participants who were required to perform bench-stepping

protocols to induce muscle damage. Participants in the other three studies were all physically active but not highly trained and were required to perform eccentric cycling (Walsh et al., 2001), barbell squatting (Moysi et al., 2005) and countermovement jumps (Twist & Eston, 2009) in order to induce muscle damage. No change in $\dot{V}O_2$ measures was reported in any of the aforementioned studies during sub-maximal constant load cycling. Furthermore, it has been reported that peak $\dot{V}O_2$ measured during incremental cycling to exhaustion was unaffected by a prior bout of eccentric, bench-stepping exercise (Gleeson et al., 1998). The observation that cycling is not susceptible to the potentially confounding influence of altered kinematics makes it a particularly attractive model with which to examine the effect of EIMD on the human response to dynamic exercise. The fixed geometry of a cycle ergometer ensures that hip and knee angles remain relatively constant during exhaustive cycling exercise despite the development of significant localised muscle fatigue (Dingwell et al., 2008). In contrast, reductions of up to 7% in the range of motion of the knee joint have been reported during running following the downhill running. (Chen et al., 2007b).

2.7.2 Oxygen uptake kinetics and muscle oxygenation

While measurements of $\dot{V}O_2$ at constant sub-maximal speeds or loads provide information relating to the energy cost of locomotion, the rate at which O_2 uptake increases at exercise onset ($\dot{V}O_2$ kinetics) can provide information pertaining to the balance of O_2 delivery to O_2 utilisation in active skeletal muscle. Schneider and colleagues (2007) were the first to investigate the influence of EIMD on O_2 uptake kinetics. Nine untrained participants each performed square-wave transitions from unloaded to heavy intensity cycling before and 48

and 72 hours after completing 30 min of bench-stepping exercise. The heavy intensity exercise was determined as the work load equivalent to 40% of the difference (40 % Δ) between the power output achieved at the gas exchange threshold (GET) and that achieved at peak $\dot{V}O_2$. The phase II $\dot{V}O_2$ kinetics were unaltered by the prior eccentric exercise indicating that EIMD did not compromise oxidative function or alter the matching of O_2 delivery to O_2 utilisation in the active muscle tissue. In addition, the unchanged slow component indicated that the O_2 cost of cycling at 40 % Δ was not altered. These findings led the authors to speculate that they had not induced sufficiently severe muscle damage to elicit changes in $\dot{V}O_2$ kinetics. While muscle oxygenation was not measured in this investigation, near-infrared spectroscopy (NIRS) can facilitate the assessment of muscle oxygenation and can therefore be used to non-invasively determine the dynamic balance between O_2 delivery and O_2 utilisation.

Walsh et al. (2001) examined the kinetics of O_2 utilisation and re-oxygenation during ischemia and reperfusion at rest before and 2 days after 30 min of eccentric cycling. No change was reported in O_2 utilisation or local O_2 transport with the authors concluding that muscle oxidative function at rest was not impaired as a result of EIMD (Walsh et al., 2001). More recently Ahmadi et al. (2008) used NIRS to investigate O_2 saturation and desaturation kinetics at rest and during isometric contractions at 30, 50 and 80% MVC before and after a 40 min bout of downhill walking (-25%). In contrast to the findings of Walsh and colleagues (2001), Ahmadi et al. (2008) reported immediate and prolonged speeding of the NIRS-derived O_2 kinetics. The authors proposed that the probable mechanism for the increased O_2 saturation and desaturation may have been increased O_2

utilisation due to the requirements of energy demanding repair processes (Ahmadi et al., 2008). However the authors did concede that increases in muscle blood flow subsequent to EIMD, such as reported by Laaksonen et al. (2006), may have been responsible for the apparent speeding of O₂ saturation and desaturation kinetics (Ahmadi et al., 2008). While muscle oxygenation kinetics have been investigated at rest and during isometric contractions, to date, no study has attempted to investigate the influence of EIMD on muscle oxygenation kinetics during the performance of dynamic exercise.

2.7.3 Metabolic responses to dynamic exercise

As detailed in section 2.6.4, the reduction in resting muscle glycogen uptake following EIMD has led to speculation that increased glycolysis may be a consequence of EIMD and as such may be responsible for increases in blood lactate concentration ([La]) observed at rest (Asp et al., 1996, Asp et al., 1998) and during dynamic exercise (Braun & Dutto, 2003; Chen et al., 2007b, 2008; Gleeson et al., 1995, 1998).

Gleeson et al. (1995) were the first to report increases in immediate post exercise [La] 48 h after a 30 min bout of bench-stepping designed to induce muscle damage. Participants cycled for 15 min at an intensity equivalent to 80% $\dot{V}O_{2\max}$ 48 h after either the eccentric bench-stepping exercise or concentric uphill walking. Pre-exercise [La] was not different between the two groups but it was higher after eccentric exercise (7.5 mmol.l⁻¹) than after concentric exercise (6.0 mmol.l⁻¹). In a follow-up study, blood samples were taken before and at 2 min intervals throughout an incremental cycle test to exhaustion and demonstrated that following eccentric exercise [La] increased (Gleeson et al., 1998). In this investigation the control condition was unexercised in the period prior to completion of the incremental

exercise test and demonstrated lower [La] during and 2 min after completion of the test (Gleeson et al., 1998). These authors proposed that the higher blood [La] in the post eccentric condition reflected a higher intramuscular [La] and an increased relative contribution of anaerobic metabolism to energy production possibly arising from additional recruitment of type II muscle fibres (Gleeson et al., 1998). In support of this suggestion Braun and Dutto (2003) have reported increased blood [La] during running at 65, 75 and 85 % of peak $\dot{V}O_2$. Blood samples were taken during the 5 min rest period after each 5 min bout and were on average 0.61 mmol.l^{-1} higher 48 h after the downhill running protocol. The increase in [La], attributed to a greater reliance on glycolytic energy production, together with altered stride mechanics were suggested as contributory factors in the reduction in running economy (Braun & Dutto, 2003) (previously discussed in section 2.7.3). Similarly, Chen et al. (2007b) reported significant increases in [La] in blood samples taken 3 min after the completion of each 5 min running bout at 65, 75 and 85% $\dot{V}O_2$ peak. The authors suggested that the increases in [La] which lasted for three days following a 30 min downhill run may have reflected increased motor unit activation (Chen et al., 2007b).

However, elevations in [La] may not reflect alterations in metabolic function following EIMD. Schneider et al. (2007) reported unchanged phase II $\dot{V}O_2$ kinetics indicating that EIMD did not compromise oxidative function (see section 2.7.3) together with elevations in [La]. The difference between resting and end exercise blood [La] was higher following EIMD and was attributed to an increased lactate efflux from the active muscle due to increased membrane permeability rather than to an enhanced rate of anaerobic glycolysis (Schneider et al., 2007). An increased lactate efflux may be countered by increased muscle

blood flow following EIMD (Laaksonen et al., 2006) which could facilitate greater [La] clearance by improving transport to lactate metabolising muscle and other tissues. Increased muscle blood flow may well explain for the unchanged blood [La] reported in other studies (Hamill et al., 1991; Scott et al., 2003; Marcora & Bosio, 2007) and the reduced blood [La] reported by Moysi et al. (2005) following EIMD.

Magnetic resonance spectroscopy (MRS) can be used to measure parameters of muscle metabolic function including the dynamic changes in the ratio of inorganic phosphate to phosphocreatine (Pi/PCr) and intracellular pH. Several studies have shown a significant decrease in the resting PCr/Pi ratio following EIMD, which could be interpreted as an increase in metabolism following the muscle injury (McCully et al., 1992; Lund et al., 1998a, 1998b). However, due to fact that no commercially available ergometers are capable of functioning within a MRS, only one study has investigated possible alterations to muscle metabolism following EIMD during dynamic leg exercise inside the core of a whole body MRS. Using a hydraulic ergometer specifically designed for quadriceps exercise within a whole body MRS (Rodenburg et al., 1994), participants completed two graded concentric exercise tests before and 24 h after performing stepping exercise designed to induce EIMD (Rodenburg et al., 1995). The expected decrease in the resting PCr/Pi ratio was observed but no differences were reported in the PCr/Pi ratio during the exercise test. While these findings indicated that exercise metabolism was not altered by the stepping exercise, the lack of change in several markers of muscle damage including plasma CK activity, led the authors to conclude that muscle metabolism could feasibly be altered by more severe EIMD (Rodenburg et al., 1995). The inconclusive nature of this one set of findings suggests that further research employing MRS should be conducted in order

to more clearly illustrate the effect of EIMD on muscle metabolism during dynamic exercise.

2.7.4 Ventilatory responses to dynamic exercise

The mechanisms which control ventilation during dynamic exercise are controversial but are believed to involve elements of proportional feedback (central and carotid chemosensory) and feed-forward (central command and muscle reflex) in varying proportions (Ward, 2007). Below the lactate threshold, ventilation is involved in the regulation of the arterial partial pressure of CO₂ to near baseline levels. Above the lactate threshold bicarbonate buffering of the lactic acidosis provides an important stimulus to ventilation.

Several investigations have reported no change in ventilation following EIMD. Paschalis et al. (2005) employed the lowest intensity exercise, with participants running at “randomly selected velocities” of 133 and 200 m.min⁻¹ which were equivalent to ~ 55 and ~75% $\dot{V}O_{2\max}$ respectively (Paschalis et al., 2005). When compared to baseline measures, no change in ventilation was reported at either velocity for 4 days after completion of 120 (12 x 10) eccentric MVCs. Similarly, Scott et al. (2003) and Marcora and Bosio (2007) have reported an unaltered ventilatory response to running at intensities equivalent to a blood [La] of 2.5 mmol.l⁻¹ (ostensibly below the lactate threshold) and 70% $\dot{V}O_{2\max}$, respectively. Furthermore, these authors observed no influence of EIMD on blood [La] (Scott et al., 2003; Marcora & Bosio, 2007). Participants in the study conducted by Moysi et al. (2005) cycled at 62% $\dot{V}O_{2\max}$ for 6 min before and 48 h after completing a series of muscle damaging squats. While no overall change in minute ventilation was reported the

authors did observe increases in breathing frequency which were countered by decreases in tidal volume. An explanation for these observations and for the observation that blood [La] was decreased by 12% at 48 was not offered (Moysi et al., 2005).

Increases in ventilation in conjunction with increases in blood [La] have been reported during running at 65, 75 and 85% $\dot{V}O_{2\max}$ following EIMD (Braun & Dutto, 2003; Chen et al. 2007b, 2008) and have been associated with impaired running economy attributed to altered lower limb kinematics (see section 2.7.1). However, Gleeson et al. (1995) observed increases in both ventilation and blood [La] during cycling at 80% $\dot{V}O_{2\max}$ 48 h after eccentric exercise. Minute ventilation was increased from 6-15 min of a 15 min bout and was mainly due to a higher breathing frequency. Similarly, Schneider et al. (2007) reported increases in both ventilation and blood [La] during cycling at 40% Δ (heavy intensity exercise). While the influence of the increased lactic acidosis was considered the most likely explanation, the increased stimulation of muscle nociceptors due to increased muscle pain and the higher perception of effort reported were also proposed as potential mechanisms to drive the increase in ventilation (Gleeson et al., 1995; Schneider et al., 2007).

Schneider et al. (2007) have provided the only example of an investigation using a specific exercise domain to study the influence of EIMD on physiological responses to dynamic exercise. The exercise domain employed during an investigation is important in determining the individual physiological response to dynamic exercise. Markedly different metabolic, cardiorespiratory and perceived exertion responses are elicited from the three sub-maximal exercise intensity domains (moderate, heavy and severe). As such, it is

important that exercise intensity is accurately defined by delineating the boundaries between these domains when investigating these responses (Jones et al., 2009). The vast majority of studies investigating the influence of EIMD on the performance of dynamic exercise utilise exercise intensities determined by a percentage of maximal work capacity. This can be rather misleading due to inter-individual differences. For example, exercise at 80% $\dot{V}O_{2\max}$ (e.g. Gleeson et al., 1995), may result in one individual exercising within the heavy domain whilst another exercises within the severe domain. Future research involving the influence of EIMD on ventilatory and other human responses to dynamic exercise should endeavour to use specific exercise domains so that the interpretation of findings is not confounded by individuals exercising at different relative intensities.

Increases in ventilation following EIMD may be linked to an increased lactic acidosis, however other potential mechanisms have been investigated. In order to eliminate the influence of metabolic control factors such as [La], Hotta and colleagues (2006) investigated the ventilatory response to the first 20 s of dynamic knee extension exercise following EIMD. Knee extensions with ankle weights of approximately 2.5% body mass were repeated five to seven times. The breath by breath data were time aligned to the start of exercise, ensemble averaged and then linearly interpolated to yield 1 s data points. Ventilation at the onset of exercise was significantly elevated 2 and 7 days after eccentric exercise leading the authors to suggest that alterations in the peripheral neural reflexes contributed to the enhanced ventilatory response (Hotta et al., 2006).

2.7.5 Perception of effort during dynamic exercise

The perception of effort involves the assimilation of a number of afferent signals from various perceptual cues emanating from different body systems including the cardiorespiratory and neuromuscular systems which can be interpreted in both a feedback and feed-forward manner (Hampson et al., 2001). Cues may be peripheral, produced for example by the painful stimulation of muscle afferents following EIMD; or centrally-derived from the cardiorespiratory responses to exercise. While the damage protocol, the exercise mode and the exercise intensity may have a significant influence on many other physiological responses, the weight of evidence suggests that EIMD results in an increased sense of effort during dynamic exercise (Gleeson et al., 1995; Scott et al., 2003; Marcora & Bosio, 2007; Chen et al., 2007b, 2008; Twist & Eston, 2009).

Gleeson et al. (1995) reported an increased sense of effort during cycling at 80% $\dot{V}O_{2\max}$ 2 days after completing 30 min of bench-stepping. The higher ratings of perceived exertion (RPE) reported were believed to reflect the weakened state of the muscles, the increase in blood [La] and the sensation of muscle soreness (Gleeson et al., 1995). Increases in RPE have also been reported during running following eccentric exercise (Scott et al., 2003; Chen et al. 2007b, 2008). Scott and colleagues (2003) suggested that perceived exertion was the best indicator of physical stress as RPE is a configuration of responses resulting from an integration of signals, perceptions and experiences. In addition to the cues proposed by Gleeson and colleagues (1995), altered neural control was suggested as a possible cue to the higher perception of exertion reported following EIMD (Scott et al., 2003). Recent studies conducted by Marcora and Bosio (2007) and Twist and Eston (2009) have reported RPE responses during fixed-load submaximal exercise and time trial

performance. During running for 10 min at 70% $\dot{V}O_{2\max}$ (Marcora & Bosio, 2007) and cycling for 5 min at 60 and then 80% of maximal power output (Twist & Eston, 2009) RPE was higher following eccentric exercise. However, during both the running and cycling time trials RPE was unchanged following EIMD although time trial performances were significantly reduced. Both pairs of investigators concluded that following EIMD an altered sense of effort mediated performance. Participants reported higher RPEs when exercising at the same intensity and produced less force (slower running or cycling speed) when perceiving the same effort (Marcora & Bosio, 2007; Twist and Eston, 2009).

Several studies have demonstrated alterations in the perception of force production following eccentric exercise which persist for several days (see section 2.4.3). More recent investigations have corroborated these findings, proposing that participants were using their perception of the effort required to generate a given torque rather than the level of torque itself (Carson et al., 2002; Proske et al., 2003; Weerakkody et al., 2003a; Proske et al., 2004). These findings indicate that EIMD impairs the perception of effort or exertion rather than the ability to match a given force as participants with EIMD produce reduced force for a given perceived effort and perceive higher effort when producing the same force.

2.8 Conclusion

Direct histological analyses of muscle tissue and indirect measures of the structural and functional status of skeletal muscle following unaccustomed, eccentric exercise are well documented and have revealed substantial disruption. However, the effects of exercise-induced muscle damage on the human response to dynamic exercise have been investigated

infrequently and have produced equivocal findings. Exercise modality has been found to influence the human response following EIMD with changes observed during running more closely related to changes in kinematic parameters than changes in muscle function. Therefore cycling, which is not susceptible to the potentially confounding influence of altered kinematics, is the preferred model with which to examine the effect of EIMD on the human response to dynamic exercise. The purpose of the following studies is to investigate the influence of EIMD on various human responses during the performance of dynamic exercise. Specifically, investigations will focus on ventilatory and perceived exertion responses, muscle metabolism, muscle oxygenation kinetics and pulmonary oxygen uptake.

CHAPTER 3:
COMMON METHODS

3.1 Introduction

The experiments which comprise this thesis were conducted in the exercise physiology laboratories of the School of Sport and Health Sciences at the University of Exeter which are accredited by the British Association of Sport and Exercise Science. In Study 3 (chapter 6) ³¹P-magnetic resonance spectroscopy was conducted at the Peninsula Magnetic Resonance Research Centre at the University of Exeter. Prior to the collection of any data all participants gave written informed consent to participate in the research, which was approved by the School of Sport and Health Sciences Ethics Committee.

3.2 Familiarisation

Most of the participants recruited were members of the School of Sport and Health Sciences at the University of Exeter and were familiar with the experimental testing protocols and associated procedures. However as maximal and/or exhaustive effort was required on most visits to the laboratory, appropriate practice was conducted. Participants in all four studies were fully familiarised with the production of maximal voluntary contractions using a Biodex B-2000 isokinetic dynamometer (Biodex Corp, Shirley, NY). All participants in study 3 (chapter 6) were required to complete a familiarisation session in a 'mock MRI system'. During this session, participants practised single-legged, knee-extension exercise at a rate of 40 repetitions/min in time with a visual cue projected onto the front wall of the 'mock' scanner room. Vigorous encouragement was given to participants throughout all maximal and/or exhaustive tests to ensure a maximal effort was produced.

3.3 Eccentric muscle-damaging exercise protocol

In order to provoke muscle damage, participants in each of the four studies were required to perform 100 squats as 10 sets of 10 repetitions using a Smith machine (Figure 3.1).



Figure 3.1 A participant being guided in the correct and safe squatting technique prior to completion of 100 (Smith) squats.

This procedure involved the controlled, isotonic resistance of the external load of the bar, which was calculated to correspond to approximately 70% of each participant's body mass. This was calculated to within 5 kg of the predicted 70% body mass as the smallest available weights were 2.5 kg. Prior to commencement, participants were instructed in correct and

safe lifting technique (see appendix E for risk analysis). Before the bar was disengaged it was positioned on the participant's shoulders and feet were positioned under the bar. During the movement the participant's head was kept forward, the back straight and legs fully extended (knee = 180°). Feet were kept flat on the floor, toes pointing forward, with equal distribution of weight through fore-foot and heel. Prior to commencement, the appropriate foot position was marked on the floor with tape so that it could be reproduced in each of the ten sets of ten repetitions. The descent phase involved eccentric action of the knee extensors to lower the bar to a knee angle of just past 90°. The lifting phase involved concentric action to return the bar to the starting position. In order to facilitate the consistent production of 90° squats, a plastic metre rule was taped to the safety stop frame on the guide rod using gaffer tape to provide a visual and audible guide for the participant and investigators. When the participant achieved the required angle this could be seen in the facing mirror and heard as the metre rule contacted the base frame of the Smith machine.

During pilot work, a metronome was used in an attempt to control the speed of contraction as it has been suggested that this may influence the magnitude of damage induced (Chapman et al., 2006). However, participants struggled to maintain a set cadence of four down to one up with the metronome set at 60 beats per minute without losing form. Therefore this element of the protocol was abandoned and participants were encouraged to perform controlled decent and lifting phases at their own pace whilst maintaining the correct and safe lifting technique. After each set of ten repetitions a minimum rest period of 1 minute was allowed with participants encouraged to take as much time as they needed between sets to ensure that the correct and safe lifting technique could be maintained.

3.4 Markers of muscle damage

In a review of measurement tools used to evaluate exercise-induced muscle damage, Warren et al. (1999) proposed that maximal voluntary contraction (MVC) torque provided the best measure of muscle damage resulting from eccentric contractions. These authors were dismissive of using either changes in perceived muscle soreness or changes in the blood levels of myofibre proteins such as Creatine Kinase (CK) as they are reported to correlate poorly with the magnitude and time course of changes in muscle function (Warren et al., 1999). However, we chose to include measures of soreness and plasma CK activity in addition to those of MVC torque, in order to provide a wider physiological perspective of the damage induced. In each study these markers of muscle damage were assessed in the order listed below before and after completing the eccentric, muscle-damaging exercise in order to measure the effectiveness of the protocol.

3.4.1 Creatine Kinase activity

Plasma CK activity was assessed from fingertip capillary samples. The sample was centrifuged at 4000 RPM (2000 x g) for 5 minutes and two 20 µl samples of plasma were then added to 1 ml of a composition of reagents supplied by Randox (CK-NAC 110, Randox Laboratories Ltd., Crumlin, Co. Antrim, UK). Following 1 min incubation at 37°C and during continued incubation, absorbance at 340 nm was recorded by spectrophotometry (Jenway 6310 spectrophotometer, Jenway, Essex, UK) at 0, 1, 2 and 3min. CK values were calculated using the formula $CK(U/l) = 8095 \times \Delta \text{ absorbance } 340 \text{ nm/min}$. The mean CK value of the two samples was calculated and used for subsequent analysis. Normal serum values of 24-195 U/l are reported for men using this method (Szasz et al., 1976). The intra-

assay coefficient of variation for duplicate samples using this procedure in our laboratory was 6.6 %.

3.4.2 Perceived muscle soreness

Participants assessed the soreness of their knee extensors using a blank 0–10 visual analogue scale (VAS) (Appendix G). The VAS consisted of a 10 cm line labelled from left (no soreness) to right (worst soreness ever). After squatting to approximately 90° knee flexion with hands on hips, participants were asked to place a mark on the VAS to indicate their level of soreness. Perceived pain was then quantified by measuring the distance to the mark on the line to the nearest 0.1 cm. This procedure has been used in previous studies (Rowlands et al., 2001; Twist and Eston, 2005, 2007).

3.4.3 Isokinetic peak torque

Following familiarisation sessions, isokinetic peak torque of the knee extensors was measured using a Biodex B-2000 isokinetic dynamometer (Biodex Corp, Shirley, NY), which was calibrated prior to each data collection session in accordance with the manufacturer's guidelines (Figure 3.2).

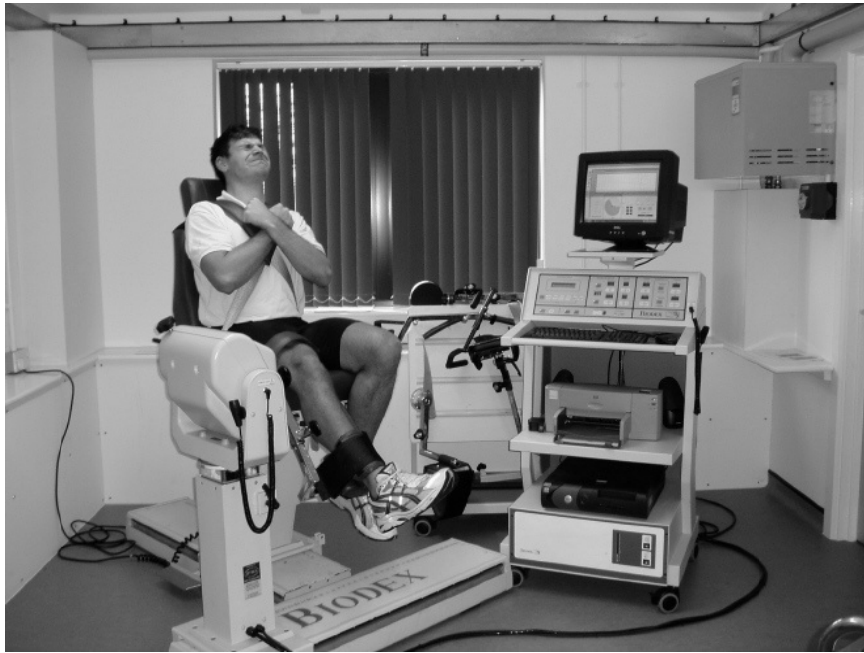


Figure 3.2 The assessment of isokinetic peak torque, using a Biodex B-2000 isokinetic dynamometer (Biodex Corp, Shirley, NY)

The initialisation, system diagnostics and calibration procedures were performed automatically by the Biodex System by pressing the Start key on the control panel. Participants completed a standardised warm-up of 2 minutes cycling at 50 W on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) followed by static stretching exercises of the knee extensor/flexor muscle groups. Participants completed a standardised warm-up of 2 minutes cycling at 50 W on the cycle ergometer followed by static stretching exercises of the knee extensor/flexor muscle groups. The participants were then seated in the isokinetic dynamometer in an upright position with the seat angle set at 85°. The ankle was secured to the input arm of the dynamometer on the tibia allowing full ankle motion, with the rotational axis of the dynamometer aligned with the lateral femoral epicondyle. All adjustments to the dynamometer were recorded and replicated in subsequent tests. The 80° range of motion

for each subject (from 90° to 10° knee flexion (full extension = 0°)) was manually established by the investigator and confirmed goniometrically. The mass of the limb was recorded by the dynamometer to enable the gravitational correction of peak torque values. The highest of five maximal voluntary contractions (MVCs) at an angular velocity of 30 deg.s⁻¹ was recorded. A 30-s rest period was allowed between contractions. Visual feedback, displaying real time force and strong verbal encouragement were used to promote maximal effort. All data were collected using the Biodex Advantage Software package and stored on the computer for subsequent analysis. The inter-test coefficient of variation for duplicate measures using this procedure in our laboratory was 4.9 %.

CHAPTER 4:
**THE EFFECT OF EXERCISE-INDUCED MUSCLE DAMAGE ON
VENTILATORY AND PERCEIVED EXERTION RESPONSES TO MODERATE
AND SEVERE INTENSITY CYCLE EXERCISE**

The contents of this chapter form the basis of the following publication:

Davies RC, Rowlands AV, Eston RG. Effect of exercise-induced muscle damage on ventilatory and perceived exertion responses to moderate and severe intensity cycle exercise. *European Journal of Applied Physiology* 2009; 107 (1):11-9.

4.1 Abstract

This study examined the effect of exercise-induced muscle damage (EIMD) on ventilatory and perceived exertion responses to cycle exercise. Ten healthy, physically active men cycled for six minutes at moderate intensity and to exhaustion at severe intensity before and 48 h after eccentric exercise (100 squats with a load corresponding to 70% of body mass). Changes in ventilation and ratings of perceived exertion (RPE) were calculated for each individual and expressed against time (moderate and severe exercise) and as a percentage of time to exhaustion (severe exercise). Ventilation increased during moderate exercise at 48 h (\dot{V}_E ; 34.5 ± 5.0 to 36.3 ± 3.8 L \cdot min $^{-1}$, $P < 0.05$) but increases in RPE were not significant. During severe exercise at 48 h, time to exhaustion (TTE) was reduced and \dot{V}_E (87.1 ± 14.1 to 93.8 ± 11.7 L \cdot min $^{-1}$) and RPE (15.5 ± 1.3 to 16.1 ± 1.4) were elevated ($P < 0.05$). When expressed as a percentage of TTE, the differences in ventilation and RPE values disappeared. Findings indicate that the augmented ventilatory response to cycle exercise following EIMD may be an important cue in informing effort perception during high intensity exercise but not during moderate intensity exercise.

4.2 Introduction

Unaccustomed exercise, particularly exercise with a high eccentric component, has a significant impact on muscle structure and function. However, the influence of EIMD on submaximal exercise performance remains equivocal. During submaximal running, reports of elevated oxygen consumption ($\dot{V}O_2$), minute ventilation (\dot{V}_E), respiratory exchange ratio (RER), blood lactate concentration ([La]) and heart rate (HR) (Braun & Dutto, 2003; Chen et al., 2007b, 2008) are countered by observations of unaltered cardiorespiratory and metabolic responses (Hamill et al., 1991; Scott et al., 2003; Paschalis et al., 2005; Marcora & Bosio, 2007).

During submaximal cycling, the $\dot{V}O_2$ response does not appear to be altered by a prior bout of eccentric, muscle-damaging exercise (Gleeson et al., 1995; Moysi et al., 2005; Schneider et al., 2007; Twist & Eston, 2009). However the influence of EIMD on other cardiorespiratory and metabolic responses remains in dispute. Elevated \dot{V}_E and HR responses have been reported during high intensity cycling 48 h after damage-inducing, bench-stepping and have been associated with increases in [La] (Gleeson et al., 1995; Schneider et al., 2007). During lower intensity exercise (62% $\dot{V}O_{2max}$) following squatting exercise, \dot{V}_E , HR and [La] appear to be unchanged (Moysi et al., 2005). Thus it would appear that mode of damage induction and the intensity and mode of exercise are important factors in determining the response to submaximal exercise with EIMD. However, irrespective of the mode of damage, exercise intensity, or exercise protocol employed, the weight of evidence supports the premise that eccentric, muscle-damaging exercise results in

an increased sense of effort (Gleeson et al., 1995; Scott et al., 2003; Marcora & Bosio, 2007; Chen et al., 2007b, 2008; Twist & Eston, 2009).

Impaired force-generation consequential to EIMD results from reduced neural input to the muscle which serves as a protective mechanism to prevent further injury (Proske et al., 2004). Similarly, the higher ratings of perceived exertion (RPEs) reported during sub-maximal exercise following EIMD may also contribute to a putative centrally-mediated protective system. The perception of effort involves the assimilation of numerous afferent signals from a range of perceptual cues emanating from various body systems including the cardiorespiratory and neuromuscular systems, which can be interpreted in both a feedback and feed-forward manner (Hampson et al., 2001).

The cues which inform the perceptual response to exercise may arise from central or peripheral sensations. Central cues, which reflect the aerobic demands of the exercise (Åstrand & Ryhming, 1954), are derived from the cardiorespiratory system, whereas peripheral cues include local muscle sensations (such as painful muscles) and sensations produced by the stimulation of mechanoreceptors and chemoreceptors (Watt & Grove, 1993; Robertson & Noble, 1997). The influence of central (cardiorespiratory) cues is less important than that of local sensations, particularly at lower exercise intensities (Mihevic, 1981; Hampson et al., 2001). Thus, the perception of exertion reported during exercise with EIMD may be differentially influenced by central and peripheral cues dependent on the exercise intensity.

The exercise domain employed during investigations is important in determining the cardiorespiratory and perceived exertion responses. The three sub-maximal exercise intensity domains (moderate, heavy and severe) elicit markedly different metabolic, physiological and perceived exertion responses. The physiological events which separate the different exercise domains are the lactate threshold, which marks the transition from the moderate to heavy intensity exercise and the maximum lactate steady-state or critical power, which marks the transition from heavy to severe intensity exercise. Thus, it is important that exercise intensity is accurately defined by delineating the boundaries between these domains when investigating such responses (Jones et al., 2009). Investigations that utilise exercise intensity as determined by a percentage of maximal work capacity can be misleading due to inter-individual differences. For example, exercise at 80% $\dot{V}O_{2max}$, an intensity typically used to investigate the influence of EIMD on submaximal exercise performance (e.g. Gleeson et al., 1995; Twist & Eston, 2009), may result in one individual exercising within the *heavy* domain while another may be within the *severe* domain.

Therefore, the purpose of this study was to investigate changes in ventilatory and perceived exertion responses to cycling with and without exercise-induced muscle damage. Specifically, to investigate these changes during 6 minutes of moderate intensity cycle exercise and exhaustive, severe intensity cycle exercise. We hypothesised that eccentric muscle-damaging exercise would elevate the ventilatory response to severe intensity exercise but would not alter the response during moderate intensity exercise. In addition, we predicted that following prior eccentric exercise the perceived exertion responses to

cycle exercise at both exercise intensities would be elevated and that the scalar time property of perceived exertion during exercise to exhaustion would not be affected.

4.3 Method

Participants

Ten healthy, physically active men volunteered to participate in the study. All were asymptomatic of illness and pre-existing injuries and had not participated in any resistance training of the lower limbs for at least six months prior to assessment. Their characteristics are shown in Table 4.1. Participants gave written informed consent to participate in the research, which was approved by the Ethics Committee of the School of Sport and Health Sciences at the University of Exeter (See appendices B, C and D for exemplar participant information sheet, participant consent form and ethical approval certificate).

Table 4.1 Participant characteristics ($N = 10$).

Characteristic	Mean (\pm SD)
Age (yrs)	21.5 (\pm 1.2)
Height (m)	1.82 (\pm 0.06)
Mass (kg)	79.8 (\pm 8.2)
Max $\dot{V}O_2$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	48 (\pm 3)
Max HR ($\text{beats}\cdot\text{min}^{-1}$)	194 (\pm 6)
Max WR (W)	353 (\pm 30)
WR at GET (W)	120 (\pm 14)
Moderate WR (W)	96 (\pm 11)
Severe WR (W)	283 (\pm 24)

Maximal (max), gas exchange threshold (GET) and other work rate (WR) values are those measured during the ramp test.

Participants were requested not to take any anti-inflammatory drugs for the duration of the study and were instructed to report to the laboratory in a rested state, having completed no strenuous exercise in the preceding 24 h. Changes in $\dot{V}O_2$ kinetics and deoxyhaemoglobin kinetics due to EIMD were assessed in a sub-sample and this data has been reported elsewhere (Chapter 7).

Procedures

Exercise testing

Participants were required to visit the laboratory at the same time of day (± 1 h) on four occasions over a 3-week period. All testing was performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). During the first visit, after measurement of height and body mass (SECA, Hamburg, Germany) participants completed an incremental (ramp) cycle exercise test to exhaustion to establish maximal oxygen uptake ($\dot{V}O_{2\max}$) and gas-exchange threshold (GET) and to establish future work intensities (Table 4.1). Participants cycled at a self-selected pedal rate (between 70 and 90 rpm) and this pedal rate along with the saddle and handlebar height and configuration were recorded and reproduced in subsequent tests. The ramp test consisted of 4 minutes of baseline cycling at 0 W followed by a continuous increase in work rate of 1 W every 2 s (i.e. $30 \text{ W}\cdot\text{min}^{-1}$) until the subject was unable to continue. The $\dot{V}O_{2\max}$ was identified as the highest 30-s mean value recorded prior to the participant's volitional termination of the test. The GET was determined independently by two experienced reviewers using the V-slope method (Beaver et al., 1986). This involves visual inspection of individual plots of $\dot{V}CO_2$ against $\dot{V}O_2$ to establish the first disproportionate increase in $\dot{V}CO_2$. The work rates that required 80% of the GET (moderate exercise) and 70% of the difference (Δ)

between the GET and $\dot{V}O_{2\max}$ (severe exercise) were calculated, with account taken of the mean response time of the $\dot{V}O_2$ adaptation to ramp exercise (approximately 2/3 of the ramp rate i.e. minus 20 W) (Whipp et al., 1981). On the second and fourth visits, before and 48 h after eccentric exercise, respectively, participants cycled at a self-selected pedal rate (between 70 and 90 rpm) for six min at a constant work rate (WR) of 80% GET (moderate). After six min rest, participants cycled at a constant work rate of 70% Δ (severe intensity) and continued until volitional termination of the test at exhaustion. Participants received strong verbal encouragement to continue exercising for as long as possible in all exhaustive tests.

Eccentric exercise

On the third visit participants completed the eccentric, muscle-damaging exercise protocol which comprised 100 (Smith) squats performed as 10 sets of 10 repetitions. The load on the bar was calculated to correspond to 70% of each participant's body mass. For further details of these procedures please refer to Chapter 3.

Measurements

Markers of muscle damage

Markers of muscle damage (muscle soreness and isokinetic peak torque) were measured immediately before and then 30 minutes and 48 hours after eccentric exercise. In addition plasma creatine kinase (CK) activity was assessed immediately before and then 30 minutes, 24 and 48 hours after eccentric exercise. Due to availability at 24 h, only 8 participants were measured for CK activity.

Perceived soreness of the knee extensors was assessed using a blank 0–10 visual analogue scale (VAS). The VAS consisted of a 10 cm line labelled from left (no soreness) to right (worst soreness ever). Participants squatted to 90° knee flexion with hands on hips and then placed a mark on the VAS to indicate their level of soreness. Perceived pain was then quantified by measuring the distance from 0 to the mark on the line to the nearest 0.1 cm.

Plasma CK activity was assessed from fingertip capillary samples. The sample was centrifuged at 4000 RPM (2000 x g) for 5 minutes and two 20 µl samples of plasma were then added to 1 ml of reagents (Randox CK-NAC 110, Randox Laboratories Ltd., Crumlin, Co. Antrim, UK). The solution was then incubated at 37°C and absorbance at 340 nm was recorded by spectrophotometry (Jenway 6310 spectrophotometer, Jenway, Essex, UK) at 1, 2, 3 and 4 min. CK values were calculated using the formula $CK(U/l) = 8095 \times \Delta \text{absorbance } 340 \text{ nm/min}$. The mean CK value of the two samples was calculated and used for subsequent analysis. Normal serum values of 24-195 U/l are reported for men using this method (Szasz, 1976). The intra-assay coefficient of variation for duplicate samples using this procedure in our laboratory was 6.6 %.

Isokinetic peak torque was measured using a Biodex B-2000 isokinetic dynamometer (Biodex Corp, Shirley, NY), which was calibrated prior to each data collection session in accordance with the manufacturer's guidelines. Following familiarization sessions, participants performed five maximal voluntary contractions (MVCs) at 30 deg·s⁻¹ with a rest period of 30 s between contractions. The inter-test coefficient of variation for duplicate measures using this procedure in our laboratory was 4.9 %. Visual feedback, displaying

real time force, was used to encourage maximal effort. For further details of these procedures please refer to Chapter 3.

Exercise test measures

Pulmonary gas exchange was measured breath-by-breath via an online gas analysis system (Cortex MetaMax 3B, Biophysik, Leipzig, Germany) throughout all exercise tests. Changes in breathing frequency (f_R), minute ventilation (\dot{V}_E), oxygen uptake ($\dot{V}O_2$) and $\dot{V}_E/\dot{V}O_2$ were recorded continuously during the tests via the Cortex Metasoft 3.1 software. The system was calibrated prior to every test in accordance with manufacturer's guidelines against known concentrations of cylinder gases (15% oxygen, 5% carbon dioxide) and a 3-l calibration syringe (for flow volume). Heart rate (HR) was monitored using a wireless chest strap telemetry system (Polar Electro T31, Kempele, Finland) and recorded continuously via a link to the Cortex gas analysis system in all exercise tests.

Participants were familiarised with Borg's 6-20 Rating of Perceived Exertion (RPE) Scale and provided with standardised instructions on how to employ the scale (Borg, 1998). Participants were encouraged to focus on their overall perception of exertion when reporting their RPE, which were recorded during the last 15 seconds of each minute of all exercise tests (Figure 4.1).

The blood lactate response was assessed from fingertip blood samples collected immediately before and immediately after the moderate and severe intensity exercise bouts. Samples were collected into a Lithium heparin microvette (CB300, Sarstedt AG & Co.,

Nümbrecht, Germany) and were subsequently analysed for blood lactate concentration using a YSI 2300 STAT plus analyzer (Yellow Springs, Ohio, USA).



Figure 4.1 A participant reporting his RPE during cycle exercise using the Borg 6-20 RPE Scale.

Statistical analysis

Markers of muscle damage

Changes in the markers of muscle damage (isokinetic peak torque, perceived muscle soreness and creatine kinase activity) were analysed using a series of one way repeated measures analyses of variance (ANOVAs). Data were initially checked for assumptions of normality and as the CK activity data were not normally distributed; these values were log-

transformed prior to statistical analysis. Following transformation CK activity data were normally distributed (see appendix H).

Exercise test measures

Changes in f_{R_2} , \dot{V}_E , $\dot{V}O_2$, HR, $\dot{V}_E/\dot{V}O_2$ and RPE were calculated for each individual and expressed against time (minute values) for both moderate and severe intensity exercise, and in the case of severe exercise, as a % of time to exhaustion (% time). These values were analysed via a series of two-factor (test x time) fully repeated measures ANOVAs. Where assumptions of sphericity were violated ($P < .05$) the Greenhouse-Geisser (GG) correction factor was applied to adjust the degrees of freedom. Where a significant test x time interaction was observed, post hoc Tukey tests modified for repeated measures (Stevens, 2002) were run to determine where significant differences occurred. Paired t-tests were used to determine significant differences in blood lactate concentration [La] and time to exhaustion during severe intensity exercise pre- and post-eccentric exercise. All data were analysed using the statistical software package SPSS for Windows (version 13). Statistical significance was set at 0.05. A P -value of between 0.05 and 0.10 was considered a trend.

4.4 Results

Markers of muscle damage

The eccentric exercise was effective in provoking significant changes in all markers of muscle damage. Table 4.2 shows changes in isokinetic peak torque, muscle soreness and CK activity before and after eccentric exercise. Isokinetic peak torque (30 deg.s^{-1}) decreased to 86% of pre damage values at 30 min recovering to 89% at 48h ($F_{(2, 18)} = 13.37$ $P < 0.001$). Significant soreness was reported 30 min after eccentric exercise with the

highest values reported at 48 h ($F_{(2, 18)} = 71.49$ $P < 0.001$). CK activity increased after eccentric exercise, with the highest values observed at 24 h ($F_{GG(1.4, 9.8)} = 13.13$ $P < 0.05$).

Table 4.2 Changes in markers of muscle damage. Mean (\pm SD) values before (pre) and 30min and 48h after eccentric exercise

Measured variable	pre	30 min	24 h	48 h
CK activity (U/l)	178 \pm 129	244 \pm 181	794 \pm 690*	88 \pm 217
Soreness	0.5 (0.5)	5.4 (1.6)*	n/a	6.8 (1.6)*
Peak Torque (Nm) 30 deg.s⁻¹	279(45)	239 (28)*	n/a	248 (35)*

$N = 10$, Soreness and Peak Torque; $N = 8$, CK activity* Significantly different from pre value ($P < 0.05$).

Exercise test measures

Moderate intensity exercise

Table 4.3 shows mean (\pm SD) changes f_{R_2} , \dot{V}_E , $\dot{V}O_2$, HR, $\dot{V}_E/\dot{V}O_2$ and RPE respectively during cycling at moderate intensity (80% GET) pre and 48 h post eccentric exercise. Main effects for test for f_R ($F_{(1, 9)} = 7.29$, $P = 0.024$), \dot{V}_E ($F_{(1, 9)} = 6.36$, $P = 0.033$) and HR ($F_{(1, 9)} = 12.52$, $P = 0.006$) indicated an increase in values following eccentric exercise. There were no significant differences in $\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$ or RPE as a result of eccentric exercise ($P > 0.05$). Similarly, there were no significant differences in pre and post exercise blood lactate concentration before and after eccentric exercise (pre: 1.13 ± 0.43 , 1.08 ± 0.29 and post: 0.98 ± 0.21 , 1.10 ± 0.28 mmol.l⁻¹ before and after eccentric exercise, respectively) ($P > 0.05$).

Table 4.3 Changes in f_R , \dot{V}_E , $\dot{V}O_2$, HR, $\dot{V}_E/\dot{V}O_2$ and RPE during moderate intensity exercise. Mean (\pm SD) values before (pre) and 48h after (post) eccentric exercise.

	f_R (breaths min^{-1})	\dot{V}_E (L min^{-1})	$\dot{V}O_2$ (ml $\text{kg}^{-1}\cdot\text{min}^{-1}$)	HR (Beats min^{-1})	$\dot{V}_E/\dot{V}O_2$	RPE
Pre	20.6 (\pm 3.5)	34.5 (\pm 5.0)	19.9 (\pm 0.9)	109 (\pm 7)	21.8 (\pm 1.6)	9.9 (\pm 1.7)
Post	21.8 (\pm 3.2)*	36.3 (\pm 3.8)*	20.1 (\pm 1.8)	116 (\pm 8)*	22.7 (\pm 1.2)	10.6 (\pm 1.6)

* Significantly different from pre value ($P < 0.05$) N = 10

Severe intensity exercise to exhaustion

As expected, time to exhaustion was significantly reduced following eccentric exercise (7.33 ± 2.26 , 6.37 ± 2.16 min pre- and post-eccentric exercise, respectively) ($t_{(9)} = 2.64$, $P = 0.027$). Before eccentric exercise all 10 participants completed a minimum of 5 min severe intensity. However, 48 h after eccentric exercise the minimum time achieved by all participants was reduced to 4 min. Pre-exercise [La] was unchanged, but end-exercise [La] was significantly lower following eccentric exercise ($t_{(9)} = 3.31$, $P = 0.009$) (pre-exercise: 0.90 ± 0.34 , 0.79 ± 0.34 and end exercise: 9.33 ± 1.73 , 8.45 ± 1.51 mmol.l⁻¹ before and after eccentric exercise, respectively).

Minute-by-minute measures

In order to investigate minute-by-minute changes in f_R , \dot{V}_E , $\dot{V}O_2$, HR, $\dot{V}_E/\dot{V}O_2$ and RPE, the first 4 minutes of data were analysed. Figure 4.2 a–f shows minute-by-minute changes for minutes 1–4 in f_R , \dot{V}_E , $\dot{V}O_2$, HR, $\dot{V}_E/\dot{V}O_2$ and RPE respectively during cycling at 70% Δ pre and post eccentric exercise. A main effect for time was observed for all

variables ($P < 0.05$). Main effects for test for f_R ($F_{(1, 9)} = 10.53, P = 0.010$), \dot{V}_E ($F_{(1, 9)} = 9.50, P = 0.013$), $\dot{V}_E/\dot{V}O_2$ ($F_{(1, 9)} = 8.47, P = 0.017$), and RPE ($F_{(1, 9)} = 8.15, P = 0.019$) and a trend for HR ($F_{(1, 9)} = 4.43, P = 0.065$) showed that values had increased following eccentric exercise. There was an interaction (test*time) for f_R ($F_{GG(1.3, 11.3)} = 5.25, P = 0.036$) and \dot{V}_E ($F_{(3, 27)} = 3.19, P = .039$). Post hoc tests indicated that following eccentric exercise, f_R and \dot{V}_E were significantly higher for all but the first minute of exercise. There were no significant differences in $\dot{V}O_2$ ($P > 0.05$).

Percentage time values

Figure 4.3 a–f shows % time changes in f_R , \dot{V}_E , $\dot{V}O_2$, HR, $\dot{V}_E/\dot{V}O_2$ and RPE respectively during cycling at 70% Δ pre and post eccentric exercise. When expressed as a % of time to volitional exhaustion, the main effects for time remained but the main effects for test for f_R , \dot{V}_E , HR and RPE disappeared. However, there was a main effect for test for $\dot{V}O_2$ ($F_{(1, 9)} = 5.25, P = 0.048$) which showed that values had decreased following eccentric exercise. This was accompanied by an increase in $\dot{V}_E/\dot{V}O_2$ (main effect, $F_{(1, 9)} = 5.17, P = 0.049$).

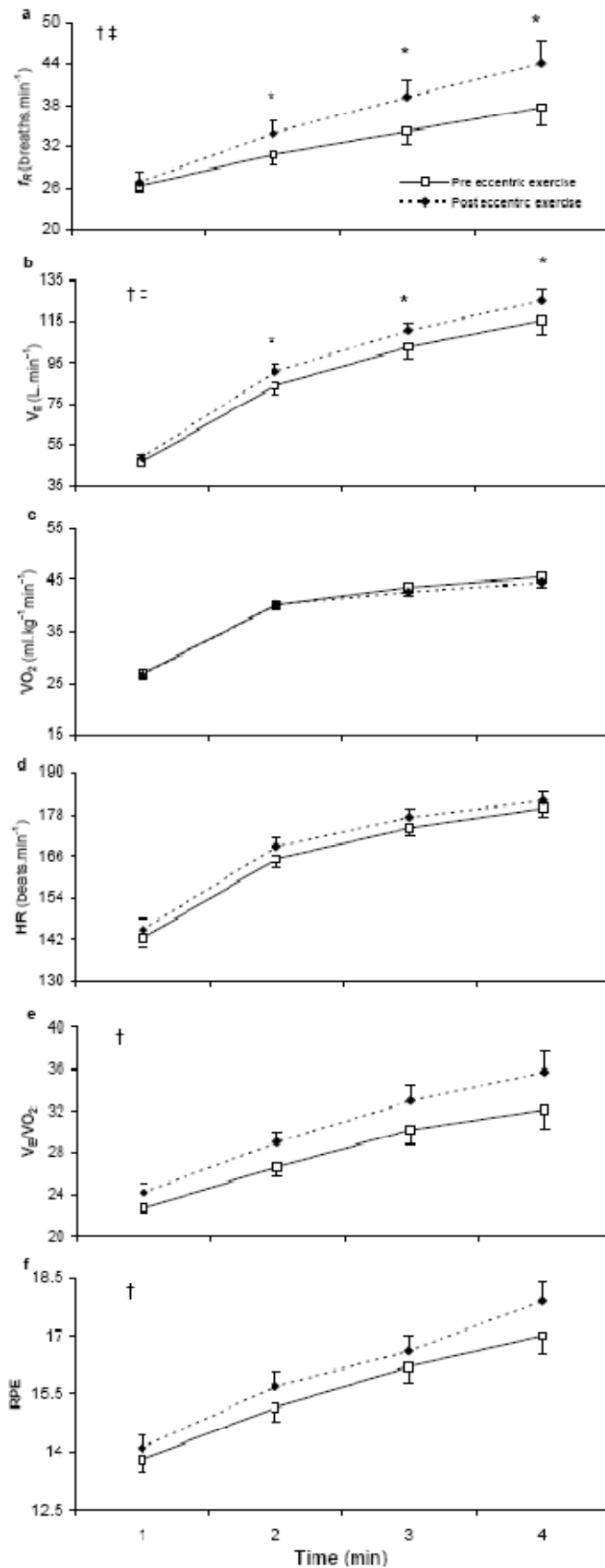


Figure 4.2

Minute values (severe intensity exercise). Minute-by-minute changes in **a**: breathing frequency (f_R), **b**: minute ventilation (\dot{V}_E), **c**: oxygen uptake ($\dot{V}O_2$), **d**: HR, **e**: $\dot{V}_E/\dot{V}O_2$ and **f**: RPE during cycling at 70% Δ pre- and 48h post-eccentric exercise.

Values are mean (\pm SEM). Significant main effect for time for all measures ($P < 0.05$).

† Significant main effect for test ($P < 0.05$).

‡ Significant interaction effect (test*time) ($P < 0.05$).

* Significantly different from pre value ($P < 0.05$).

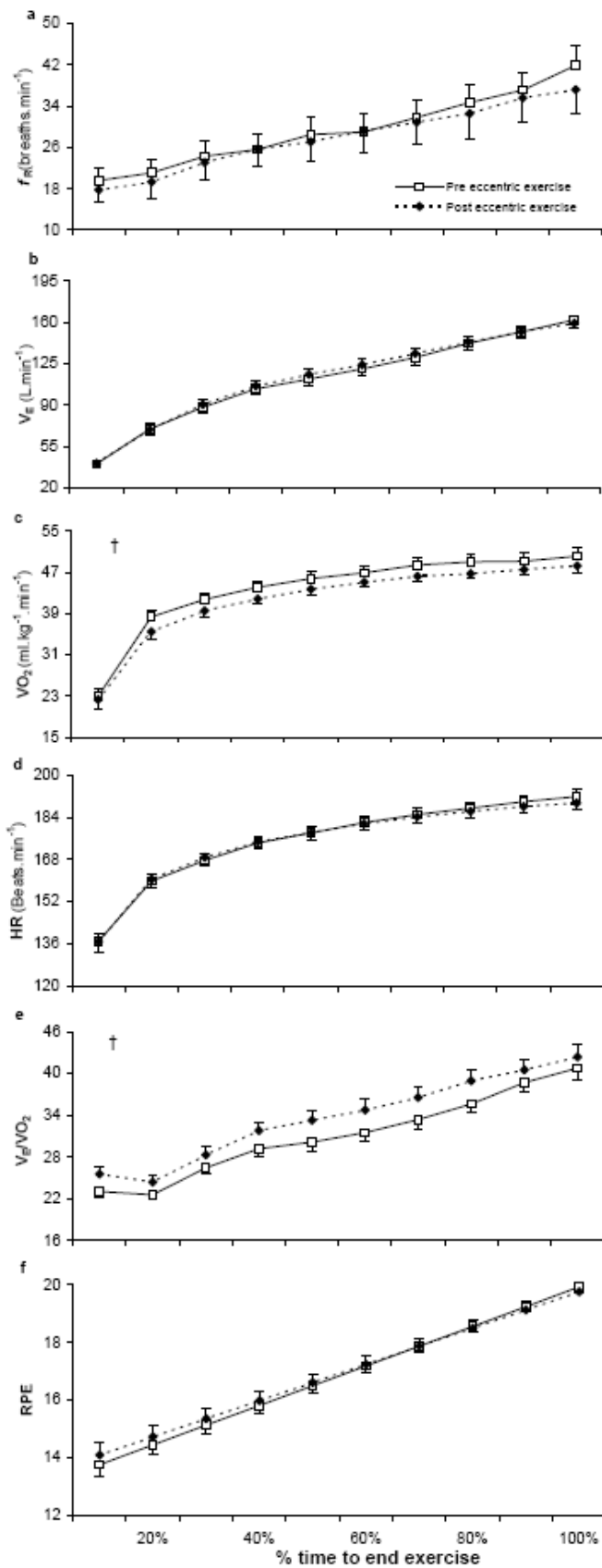


Figure 4.3

Percentage time values (severe intensity exercise). Changes in **a**: breathing frequency (f_R), **b**: minute ventilation (\dot{V}_E), **c**: oxygen uptake ($\dot{V}O_2$), **d**: HR, **e**: $\dot{V}_E/\dot{V}O_2$ and **f**: RPE during cycling at 70% Δ pre and 48h post-eccentric exercise set against % time to volitional exhaustion.

Values are mean (\pm SEM). Significant effect for time for all measures ($P < 0.05$).

† Significant main effect for test ($P < 0.05$).

‡ Significant interaction effect (test*time) ($P < 0.05$).

* Significantly different from pre value ($P < 0.05$)

4.5 Discussion

This investigation demonstrates that a prior bout of eccentric, muscle-damaging exercise augments the ventilatory response not only during severe intensity (70% Δ) cycle exercise but also during moderate intensity (80% GET) cycle exercise. In addition, this study shows that the perception of exertion is elevated during severe intensity exercise but appears to be unaffected by a prior bout of eccentric exercise during moderate exercise.

Markers of muscle damage

As anticipated, the squatting protocol was effective in provoking changes in the markers of muscle damage in all participants. The significant decreases in peak torque observed following eccentric exercise concur with previously reported findings (Byrne et al., 2001). Perceived muscle soreness was elevated above baseline measures at 30 min after the eccentric exercise with the highest values reported at 48 h. These findings are consistent with the previously reported characteristic temporal profile of increased muscle soreness following eccentrically-biased exercise (e.g. Twist & Eston, 2005). As expected, the time course of the CK response did not reflect the alterations in muscle function or perceived soreness (Warren et al., 1999). However, the CK efflux, which was greatest at 24 h, does provide indirect evidence of increased myocyte membrane permeability (Allen et al., 1995). A rapid rise and peak in CK activity similar to that observed herein has previously been observed following a similar eccentric exercise protocol (100 squats @ 70% body mass, Byrne & Eston, 2002a).

Exercise test measures

The elevated ventilatory response to severe intensity cycle exercise observed in this study concurs with previously reported observations during high-intensity, fixed-load cycle

exercise following muscle-damaging exercise (Gleeson et al., 1995; Schneider et al., 2007). However, the observation that \dot{V}_E and f_R were also increased during moderate intensity exercise (<GET) was unexpected. Using a similar squatting protocol, which produced comparable levels of strength loss (-14% at 48 h) and an exercise intensity of 62% $\dot{V}O_{2max}$ Moysi et al. (2005) reported no change in ventilation or HR. In contrast, increases in both ventilation and HR were observed in the present study, despite the fact that subjects exercised a considerably lower intensity, 80%GET, equivalent to ~27% $\dot{V}O_{2max}$ for these individuals.

The elevated ventilatory response observed whilst exercising with EIMD or DOMS has been associated with alterations in metabolic factors. Specifically, it has been assumed that an increase in [La] contributes to an augmented ventilatory response. The elevated [La] may result from an increased dependence on type II fibres and a corresponding shift to increased glycolytic energy production following muscle-damaging exercise (Braun & Dutto, 2003; Chen et al., 2007b; Gleeson et al., 1995). However, no such change in [La] was observed in the current study at either exercise intensity. Importantly, the observation that the ventilatory response following EIMD is higher during exercise below GET suggests that the altered exercise response may not be due to changes in metabolic factors. In support of this proposition, Schneider et al. (2007) observed that phase II $\dot{V}O_2$ kinetics were not altered by DOMS and concluded that elevated [La] did not result from altered oxidative function. Rather, these authors suggested that higher levels of [La] arose from an increased rate of lactate efflux from damaged myocytes due to increased membrane permeability. However, the higher rate of lactate efflux may be countered by enhanced clearance facilitated by increased muscle blood flow (Laaksonen et al., 2006) and

accordingly elicit an unchanged [La] response. Whilst the putative influence of [La] on the ventilatory response should not be dismissed; in light of the findings of this study, other potential stimuli demand consideration.

The increases in \dot{V}_E and f_R observed in the present study during moderate and severe intensities and in $\dot{V}_E/\dot{V}O_2$ during severe intensity may be attributed to alterations in neural factors. The complex mechanisms involved in ventilatory control during exercise involve a combination of central command and afferent feedback but these are poorly understood. However, ventilation is known to increase in response to painful stimulation (Haouzi et al., 2004). Thus the local muscle pain which occurs as a result of eccentric exercise, such as observed in the present study, would be expected to have a stimulatory effect on ventilation. It has been proposed that group III and IV afferent fibres located in and around the blood vessels of exercising muscle are involved in modulating the ventilatory response (Haouzi et al., 2004). Distension of these blood vessels, such as the changes in the capillary lumen shape observed by Kano et al. (2005) following eccentric exercise, would provoke a discharge from the afferent fibres leading to an increase in ventilation. Thus, neural monitoring of peripheral vascular and local muscular events may, in part, account for the augmented ventilatory response observed. Hotta et al. (2006) suggested that changes in neural factors contribute not only to an enhanced ventilatory response but also to alterations in force generation. Following eccentric exercise increased motor unit activation may be necessary in order to achieve a given sub-maximal force (Semmler et al., 2007). Similarly, a greater sense of effort is reported when producing a specific force following eccentric exercise (Proske et al., 2004).

Consistent with these observations, subjects in the present study reported higher ratings of perceived exertion (RPE) during fixed-load, severe intensity cycle exercise following eccentric exercise, although RPE appeared to be unchanged during moderate exercise. Jameson and Ring (2000) have suggested that during cycle exercise, ratings of perceived exertion are based on a combination of leg muscle pain and feelings of breathlessness. Thus the increased leg muscle soreness experienced by participants in this study following the eccentric exercise is likely to have provided an important peripheral cue to inform the RPE response. Similarly, the increased ventilatory response may have provided an important central cue. Although the relative contribution of various central and peripheral cues is poorly understood, central cues may be less important than peripheral cues, particularly during low intensity exercise (Mihevic, 1981; Hampson et al., 2001). It is of interest to note that the increased muscle pain and the elevated ventilatory response experienced by participants in the present study at 48h did not significantly influence the perception of exertion during moderate intensity exercise. However, during severe intensity exercise, where the central, ventilatory cues may play a more influential role in informing the perception of exertion, RPE was elevated.

The higher perception of exertion reported in the severe exercise bout at 48h may account for the reduction in time to end exercise. Elevated RPE has previously been associated with reduced time-trial performance in running (Marcora & Bosio, 2007) and in cycling (Twist & Eston, 2009). Furthermore, the amplified ventilatory response may contribute to the premature termination of exercise. Acute respiratory muscle fatigue may have increased the severity of locomotor muscle fatigue via a respiratory muscle metaboreflex

increase effort perception and further centrally-mediated reductions in motor output (Romer & Polkey, 2008).

The complex interplay of central and peripheral fatigue factors is of great importance in determining the duration of an individual participant's exercise performance. However, the participant's decision to terminate exercise is ultimately a conscious behavior based on the perception of alterations in sub-conscious homeostatic control systems (St Clair Gibson et al., 2003). As such, the perception of exertion may be considered fundamental to the individual exercise response when a participant is required to exercise to 'volitional exhaustion'. Pertinent to this is the observation that minute-by-minute differences in perceived exertion values reported during severe exercise before and after eccentric exercise are eliminated when expressed as a percentage of total exercise duration. Thus, further evidence is provided to support the proposition that there is a scalar-linear relationship between the rating of perceived exertion and exercise duration (Eston et al., 2007, Crewe et al., 2008; Faulkner et al., 2008; Joseph et al., 2008). Furthermore, the observation that the differences in \dot{V}_E and f_R are also eliminated when expressed as a proportion of time to exhaustion may provide some insight into the cues that inform the perceived exertion response when cycling at high-intensity after a prior bout of eccentric exercise.

4.6 Conclusion

This is the first study to investigate the influence of muscle-damaging exercise on the ventilatory and perceived exertion responses to cycling in specific exercise domains above and below the gas exchange threshold. Findings suggest that there is a strong link between the augmented ventilatory response to cycling following eccentric exercise and the higher rating of perceived exertion reported during exercise above GET. Furthermore additional evidence is provided to support the observation that perceived exertion scales with exercise duration during exercise to exhaustion.

CHAPTER 5:
THE EFFECT OF ECCENTRIC EXERCISE-INDUCED MUSCLE DAMAGE
ON THE GAS EXCHANGE THRESHOLD

The contents of this chapter are currently under review for publication:

Davies RC, Rowlands AV, Poole DC, Jones AM and Eston RG. Exercise-induced muscle damage dissociates the Lactate and Gas exchange thresholds. *Currently under review.*

5.1 Abstract

We tested the hypothesis that exercise-induced muscle damage (EIMD) would increase the ventilatory (\dot{V}_E) response to incremental/ramp cycle exercise (lower the gas exchange threshold), without altering the blood lactate profile thereby dissociating the gas exchange and lactate thresholds. It was considered that this intervention might provide a broader understanding of the mechanisms underlying the relationship between the GET and lactate threshold (Tlac). Ten physically active men completed maximal incremental cycle tests before (pre) and 48 h after (post) performing eccentric exercise comprising 100 squats with a load corresponding to 70% body mass. Pulmonary gas exchange was measured breath-by-breath and finger-tip blood sampled at 1-min intervals for blood [La] determination. GET occurred at a lower work rate (pre, 136 ± 27 W; post, 105 ± 19 W, $P < 0.05$) and $\dot{V}O_2$ (pre, 1.58 ± 0.26 ; post, 1.41 ± 0.14 l.min⁻¹, $P < 0.05$) after eccentric exercise. However, Tlac occurred at a similar work rate (pre, 161 ± 19 W; post, 158 ± 22 W, $P > 0.05$) and $\dot{V}O_2$ (pre, 1.90 ± 0.20 l.min⁻¹; post, 1.88 ± 0.15 l.min⁻¹, $P > 0.05$) after eccentric exercise. These findings demonstrate that EIMD dissociates the \dot{V}_E response to incremental/ramp exercise from the [La] response indicating that \dot{V}_E may be controlled by additional or altered neurogenic stimuli following eccentric exercise. Thus, due consideration of prior eccentric exercise should be made when using the gas exchange threshold to provide a non-invasive estimation of the lactate threshold.

5.2 Introduction

During dynamic incremental or ramp exercise protocols expired carbon dioxide production ($\dot{V}CO_2$) increases disproportionately as a function of $\dot{V}O_2$ above what has been termed the gas exchange threshold (GET) (Beaver et al., 1986). Traditionally, the GET has been considered to result from an obligatory increase in non-metabolic CO_2 production (and associated increases of ventilation, \dot{V}_E) due principally to plasma bicarbonate buffering of lactic acid-derived H^+ (Beaver et al., 1986; Wasserman et al., 1990). Thus, identification of the GET provides the basis for the non-invasive estimation of the lactate threshold (Tlac) (Beaver et al., 1986; Caiozzo et al., 1982; Wasserman et al., 1973, 1990). However, the \dot{V}_E and lactate responses to incremental exercise have been dissociated by various experimental and clinical conditions including exercise training (Poole & Gaesser, 1985), glycogen depletion (Hughes et al., 1982; Sabapathy et al., 2006) and McArdle's disease (Hagberg et al., 1982; Paterson et al., 1990; Riley et al., 1993). In contrast, in a recent study dichloroacetate (DCA) was reported to reduce both blood lactate [La] and \dot{V}_E during incremental exercise thereby supporting the existence of a causal link between bicarbonate buffering of lactic acidosis and increases in \dot{V}_E (Wilkerson et al., 2009). Today it is appreciated that a wide variety of humoral and neural control mechanisms (central command, afferent feedback from carotid body chemoreceptors and from contracting muscles) contribute to the \dot{V}_E response to incremental/ramp exercise (Dempsey et al., 2006; Haouzi, 2006; Haouzi et al., 2004; Waldrop & Iwamoto, 2006). While a complex integration of these mechanisms is understood to mediate the \dot{V}_E response, it is likely that under different conditions the proportional contribution of each of these might change.

During rhythmic exercise, group III and IV afferents are known to provide some \dot{V}_E drive via mechanical stimuli such as local muscular distortion, vascular distension and increased intramuscular pressure as well as via local humoral mediators including H^+ , lactate and bradykinin (Ward, 2000; Whipp et al., 1981). Eccentric exercise causes profound muscle structural and functional perturbations collectively referred to as exercise-induced muscle damage (EIMD) (Clarkson et al., 1992). EIMD augments discharge from group III and IV afferents (Avela et al., 1999; Komi, 2000; Taguchi et al., 2005) and this, rather than elevated blood lactate concentration which is not an obligatory consequence of EIMD (Twist & Eston, 2009), most probably contributes to the greater \dot{V}_E response to constant-load cycle exercise evoked by EIMD (Gleeson et al., 1995; Schneider et al., 2007; Twist & Eston, 2009) (Chapters 4).

The influence of a prior bout of eccentric exercise and the resultant EIMD on the \dot{V}_E and gas exchange responses to incremental exercise has received very limited attention. Only one previous study examined physiological responses to maximal incremental cycling; unfortunately the bench-stepping protocol utilised in that investigation produced only mild soreness (Gleeson et al., 1998). Thus, that bench-stepping protocol (Gleeson et al., 1998) may not have increased the discharge from group III and IV afferents as much as a procedure designed specifically to induce more severe EIMD. To our knowledge, the influence of eccentric muscle-damaging exercise on the GET has not been investigated previously. It is our contention that an eccentric exercise protocol such as that utilised previously in our laboratory (Chapter 4) is likely to exacerbate the \dot{V}_E response to

incremental/ramp exercise and, in so doing, potentially dissociate the \dot{V}_E , gas exchange and lactate responses.

It was the purpose of this investigation to determine the influence of EIMD on the GET and $\dot{V}_{E\max}$ responses determined during ramp exercise. Specifically, we tested the hypothesis that completion of 100 squats (performed as 10 sets of 10 reps at ~ 70% body mass) would augment the \dot{V}_E response (lower the GET) to subsequent incremental/ramp exercise in the absence of an altered blood lactate profile thereby dissociating the GET from the lactate threshold. It was speculated that this intervention might provide mechanistic insights into the relationship between lactate accumulation and ventilatory control in the presence of overt muscle damage.

5.3 Methods

Subjects

Ten healthy, physically active male subjects (age, 25 ± 7 years; mass, 80.1 ± 9.9 kg; height, 1.80 ± 0.08 m) volunteered to participate in this study. All participants were asymptomatic of illness and pre-existing injuries and had not performed any resistance training of the lower limbs within the previous 6 months. Participants provided written informed consent to participate in the study which was approved by the Ethics Committee of The School of Sport and Health Sciences at The University of Exeter (See appendices B, C and D for exemplar participant information sheet, participant consent form and ethical approval certificate).

Experimental Design

Ramp incremental exercise test

Participants performed two ramp incremental exercise tests to volitional exhaustion on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) (Figure 5.1). Each participant's preferred seat height and handlebar positions were recorded during their first test and replicated in the subsequent test. The height and mass (SECA, UK) of each participant was also recorded. Exercise tests were performed at the same time of day \pm 1 h for each participant, before and 48 h after performing eccentric, muscle-damaging exercise. Following 3 min of unloaded baseline cycling, the work rate was increased in a ramp fashion by 1 W every 2 s ($30 \text{ W}\cdot\text{min}^{-1}$) until the subject was unable to continue despite receiving strong verbal encouragement. The participants were required to maintain a pedal rate of 80 rpm during both incremental tests.



Figure 5.1 A participant performing a ramp incremental cycle exercise test.

Eccentric, muscle-damaging exercise protocol

Participants completed 100 (Smith) squats, performed as 10 sets of 10 repetitions with the load on the bar corresponding to ~70% of each participant's body mass. For further details of this procedure please refer to Chapter 3.

Measurements

Assessment of muscle damage

All indicators of muscle damage (perceived muscle soreness (using a 0-10 visual analogue scale (VAS)), creatine kinase (CK) activity and isokinetic peak torque (30 deg.s⁻¹)) were measured in the order listed, immediately before, 30 minutes after and 24 and 48 h after performing the eccentric, muscle-damaging exercise protocol. For further details of these procedures please refer to Chapter 3.

Ramp incremental exercise

Throughout the cycle exercise tests, before and 48 h after the muscle-damaging protocol, pulmonary gas exchange was measured breath-by-breath via an online gas analysis system (Cortex MetaLyzer 3B, Biophysik, Leipzig, Germany). Participants wore a nose clip and breathed through a low-dead-space, low-resistance mouthpiece. Gas exchange was measured throughout the tests using the Cortex Metasoft 3.1 software. The system was calibrated prior to every test in accordance with manufacturer's guidelines against known concentrations of cylinder gases (15% oxygen, 5% carbon dioxide) and a three litre calibration syringe (Hans Rudolph, Kansas City, USA) for gas flow. Inter-test coefficients of variation for \dot{V}_E , $\dot{V}O_2$ and $\dot{V}CO_2$ responses using this equipment in our laboratory were 4.1%, 3.1% and 4.2% (for \dot{V}_E , $\dot{V}O_2$ and $\dot{V}CO_2$, respectively).

The gas exchange data sets were blind reviewed in order to determine the $\dot{V}O_{2\text{peak}}$ and gas exchange threshold (GET). The $\dot{V}O_{2\text{peak}}$ was determined as the highest 30-s average value recorded before the participant's volitional termination of the test. The GET was determined from a cluster of measures which included 1) the first disproportionate increase in $\dot{V}CO_2$ production ($\dot{V}CO_2$) from visual inspection of individual plots of $\dot{V}CO_2$ versus $\dot{V}O_2$ (V-slope method (Beaver et al., 1986)) and 2) an increase in the ventilatory equivalent for O_2 ($\dot{V}E/\dot{V}O_2$) with no concomitant increase in the ventilatory equivalent for CO_2 ($\dot{V}E/\dot{V}CO_2$) (Caiozzo et al., 1982).

Heart rate was monitored continuously using a wireless chest strap telemetry system (Polar Electro T31, Kempele, Finland) and was recorded via a link to the Cortex gas analysis system. Finger-tip blood samples were collected into a capillary tube immediately before, after and at 1-min intervals during each incremental exercise test. Samples were subsequently analysed for whole blood lactate (YSI 2300 Sport, Yellow Springs, Ohio, USA). The blood lactate concentration [La] data sets were blind reviewed in order to determine the lactate threshold (Tlac). Tlac was determined as the $\dot{V}O_2$ associated with the work rate prior to the first clear and sustained increase in [La] above resting levels from visual inspection of individual plots of [La] vs. $\dot{V}O_2$.

Subjects were also asked to report their rating of perceived exertion (RPE) at 1-min intervals throughout the incremental tests. All subjects were familiarized with the Borg 6-

20 RPE scale and provided with standardised instructions on how to employ the scale prior to testing (see Appendix F).

Statistical analysis

Indicators of muscle damage

Changes in the indicators of muscle damage (perceived muscle soreness, creatine kinase activity and isokinetic peak torque) were analysed using a series of one-way repeated measures analyses of variance (ANOVAs). Post-hoc Tukey tests modified for repeated measures (Stevens, 2002) were run to determine where significant differences occurred. As the CK activity data were not normally distributed these values were log-transformed prior to statistical analysis (Twist & Eston, 2005) (see appendix H).

Ramp Incremental Exercise

Paired t-tests were used to determine significant differences in time to exhaustion, peak values and gas exchange threshold (GET) and lactate threshold (Tlac) values pre- and post-eccentric exercise. Further paired t-tests were used to determine significant differences in physiological responses at the $\dot{V}O_2$ corresponding to the pre-eccentric exercise GET. Pearson's product moment correlations were used to examine the relationship between GET and Tlac values. All data were analysed using the statistical software package SPSS for Windows (version 13) with statistical significance set at 0.05.

5.4 Results

Indicators of muscle damage

There were significant changes in all indicators of muscle damage following eccentric exercise. Table 5.1 shows changes in muscle soreness, CK activity and isokinetic peak torque before and after eccentric exercise.

Table 5.1 Changes in indicators of muscle damage. Mean \pm SD values before (pre) and at 30 min, 24 h and 48 h after eccentric exercise.

Measured variable	pre	30 min	24 h	48 h
Soreness (1-10 VAS)	0.4 \pm 0.4	4.4 \pm 2.2*	5.8 \pm 2.0*	6.4 \pm 3.0*
CK activity (U/L)	180 \pm 61	224 \pm 103	637 \pm 413*	350 \pm 151*
Peak Torque (N.m) 30 deg.s⁻¹	300 \pm 53	240 \pm 68*	248 \pm 62*	254 \pm 72*

* significantly different from pre-eccentric exercise value ($P < 0.05$).

Muscle soreness increased 30 min after eccentric exercise with the highest values reported at 48 h ($F_{(3, 27)} = 21.01$ $P < 0.001$). CK activity increased after eccentric exercise, with the highest values observed at 24 h ($F_{GG(1.38, 12.39)} = 9.61$ $P < 0.05$). Isokinetic peak torque (30 deg.s⁻¹) decreased by 20% at 30 min and was still 15% lower than baseline values at 48 h ($F_{(3, 27)} = 17.96$ $P < 0.001$).

Ramp Incremental Exercise

Peak values attained during the ramp tests pre- and post- eccentric exercise are shown in Table 5.2. Time to exhaustion and associated WR_{peak} values were decreased following eccentric exercise ($t_{(9)} = 2.62$, $P < 0.05$). There were no significant changes in $\dot{V}_{E peak}$,

$\dot{V}O_{2\text{ peak}}$, $\dot{V}CO_{2\text{ peak}}$, HR_{peak} , RER_{peak} , $[La]_{\text{peak}}$ or RPE_{peak} (all $P > 0.05$). Table 5.3 shows changes in variables associated with GET before and 48h after eccentric exercise. GET occurred earlier and thus at a lower WR ($t_{(9)} = 3.74$, $P = 0.005$) and $\dot{V}O_2$ ($t_{(9)} = 2.57$, $P = 0.030$). $\dot{V}CO_2$ values at GET were also significantly lower ($t_{(9)} = 2.54$, $P = 0.032$).

Table 5.2 Peak values attained during ramp exercise tests. Mean \pm SD values before (pre) and 48 h after (post) eccentric exercise

	pre	post
Time to exhaustion (s)	682 \pm 71	635 \pm 86*
WR_{Peak} (W)	341 \pm 36	318 \pm 43*
\dot{V}_E peak (l.min⁻¹)	145 \pm 22	140 \pm 35
$\dot{V}O_{2\text{ peak}}$ (l.min⁻¹)	3.34 \pm 0.38	3.27 \pm 0.35
$\dot{V}CO_{2\text{ peak}}$ (l.min⁻¹)	4.47 \pm 0.45	4.33 \pm 0.56
HR_{peak} (beats.min⁻¹)	182 \pm 12	179 \pm 13
RER_{peak}	1.35 \pm 0.10	1.33 \pm 0.12
[La]_{peak} (mmol.l⁻¹)	6.05 \pm 1.36	5.64 \pm 1.72
RPE_{peak} (Borg, 6-20 scale)	19.4 \pm 0.5	19.1 \pm 1.0

Peak minute ventilation (\dot{V}_E peak), $\dot{V}O_{2\text{ peak}}$, $\dot{V}CO_{2\text{ peak}}$, HR_{peak} and RER_{peak} were the highest 30s average values attained during the ramp exercise tests.

* significantly different from pre-eccentric exercise value ($P < 0.05$).

Table 5.3 Values attained at the gas exchange threshold (GET) during ramp exercise tests. Mean \pm SD values before (pre) and 48 h after (post) eccentric exercise

	pre	post
Time (s)	262 \pm 55	200 \pm 37*
WR (W)	136 \pm 27	105 \pm 19*
\dot{V}_E (l.min ⁻¹)	34.2 \pm 6.1	31.7 \pm 4.6
$\dot{V}O_2$ (l.min ⁻¹)	1.58 \pm 0.26	1.41 \pm 0.14*
$\dot{V}CO_2$ (l.min ⁻¹)	1.35 \pm 0.24	1.20 \pm 0.18*
[La] (mmol.l ⁻¹)	1.71 \pm 0.38	1.55 \pm 0.21
RPE (Borg 6-20 scale)	10.5 \pm 0.9	10.6 \pm 1.4

* significantly different from pre-eccentric exercise value ($P < 0.05$).

Figure 5.2 shows the $\dot{V}CO_2$ versus $\dot{V}O_2$ (panel A) and blood [lactate] (panel B) responses of a representative participant during ramp incremental exercise before and after eccentric exercise; note GET is shifted to a substantially lower $\dot{V}O_2$ after eccentric exercise whereas T_{lac} is essentially unchanged. There were no significant differences in \dot{V}_E , [La] or RPE at GET before and 48h after eccentric exercise ($P > 0.05$).

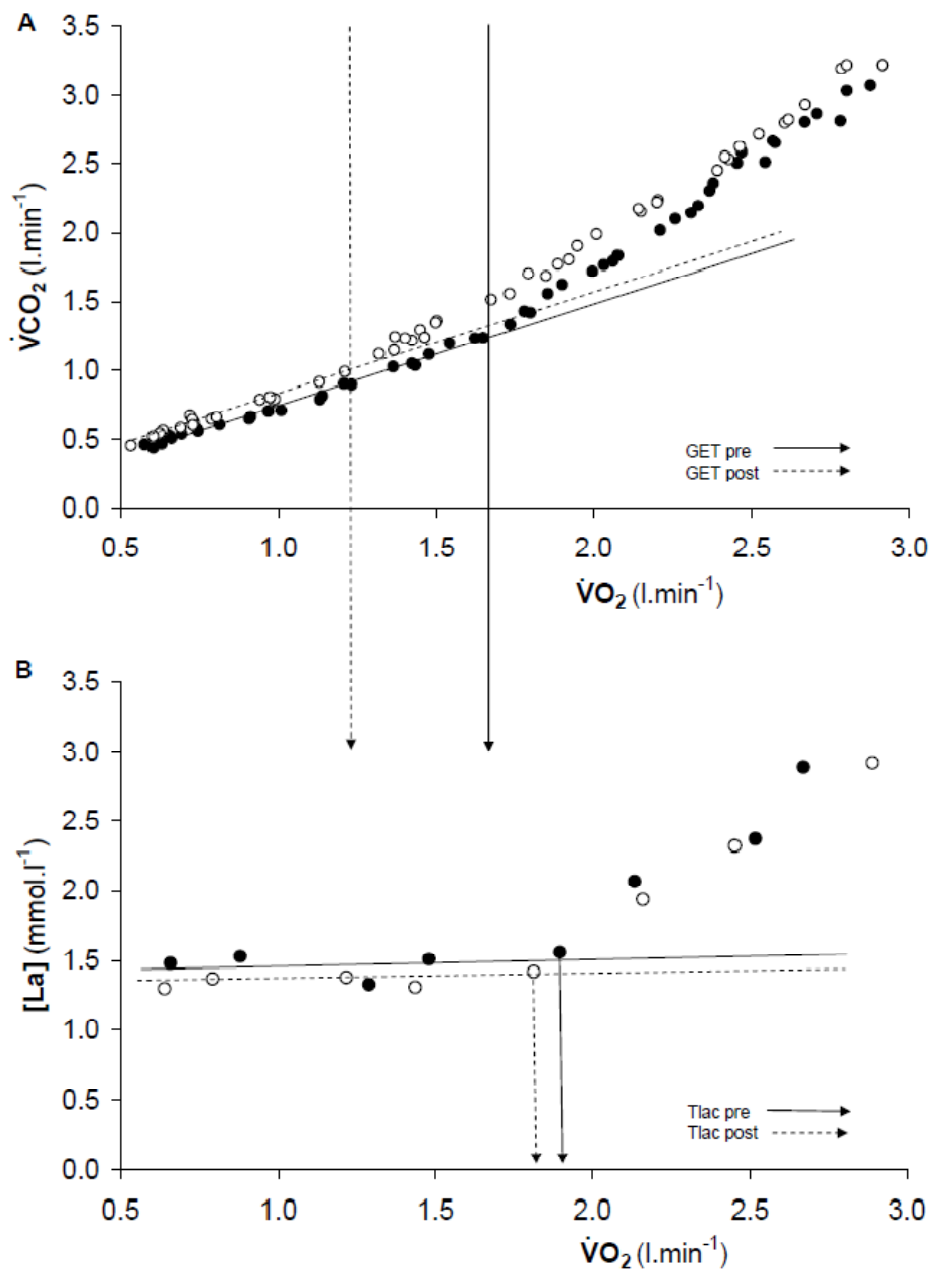


Figure 5.2 Representative response of $\dot{V}CO_2$ vs $\dot{V}O_2$ (panel A) and blood [lactate] (panel B) showing the region of interest, pre- (●) and 48 h post- (○) eccentric exercise respectively. Best-fit S1 slopes and vertical arrows indicating the gas exchange threshold (GET, panel A) and lactate threshold (Tlac, panel B) illustrate changes in the GET but not the Tlac response, pre- (solid line) and post- (dashed line) eccentric exercise.

Figure 5.3 shows RPE responses before and after eccentric exercise. The Tlac occurred at a similar work rate (pre, 161 ± 19 W; post, 158 ± 22 W, $P > 0.05$) and $\dot{V}O_2$ (pre, 1.90 ± 0.20 l.min⁻¹; post, 1.88 ± 0.15 l.min⁻¹, $P > 0.05$) after eccentric exercise. The $\dot{V}O_2$ at GET was significantly correlated with $\dot{V}O_2$ at Tlac before ($r = 0.78$, $P < 0.05$) but not after eccentric exercise ($r = 0.50$, $P > 0.05$). There was no significant correlation between changes in GET and Tlac values pre and post eccentric exercise ($r = 0.53$, $P > 0.05$).

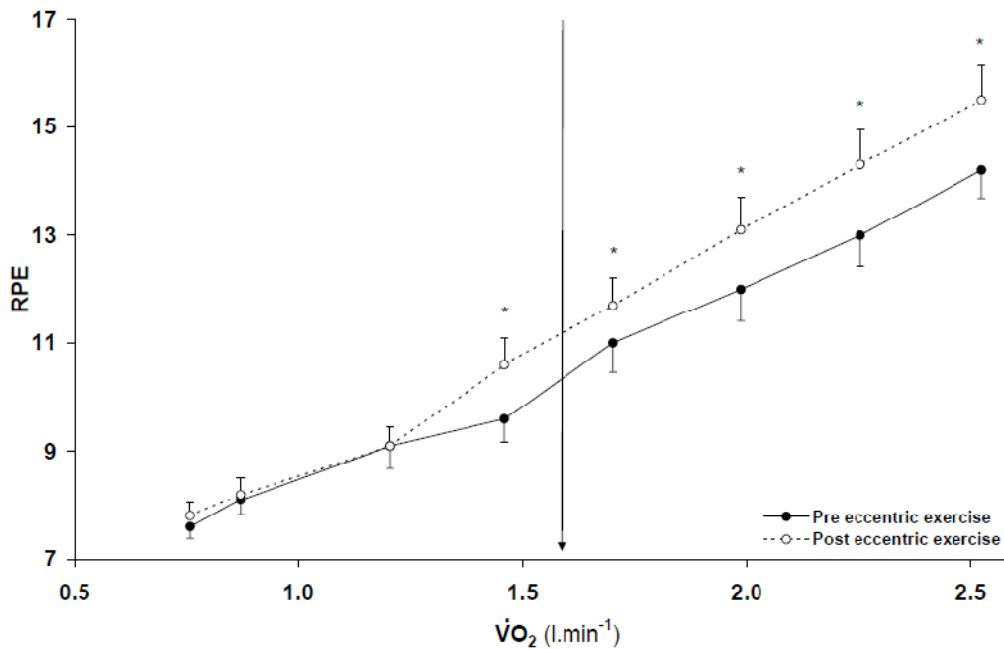


Figure 5.3 Changes in ratings of perceived exertion (RPE) as a function of $\dot{V}O_2$, pre- (●) and 48 h post- (○) eccentric exercise respectively. The vertical arrow indicates the gas exchange threshold (GET) pre-eccentric exercise. There is a 7% increase ($P < 0.05$) in RPE 48 h post eccentric exercise at the $\dot{V}O_2$ value of the pre-eccentric exercise GET.

* Significantly different from pre-eccentric exercise value ($P < 0.05$)

However, RPE increased significantly at the $\dot{V}O_2$ corresponding to the pre-eccentric exercise GET. $\dot{V}O_2$ was unchanged by the damage protocol and as anticipated, increased as a linear function of work rate before and 48 h after eccentric exercise. A comparison of responses before and 48 h after eccentric exercise at the $\dot{V}O_2$ value at which pre-eccentric exercise GET occurred revealed several significant differences (Figure 5.4). Specifically, there were increases in \dot{V}_E ($t_{(9)} = -2.88, P < 0.05$), $\dot{V}CO_2$ ($t_{(9)} = -3.31, P < 0.05$), RER ($t_{(9)} = -2.92, P < 0.05$), $\dot{V}_E/\dot{V}O_2$ ($t_{(9)} = -2.63, P < 0.05$), f_R ($t_{(9)} = -2.49, P < 0.05$), and RPE ($t_{(9)} = -2.40, P < 0.05$). There were no differences in $\dot{V}_E/\dot{V}CO_2$, V_T or $[La]$ ($P > 0.05$).

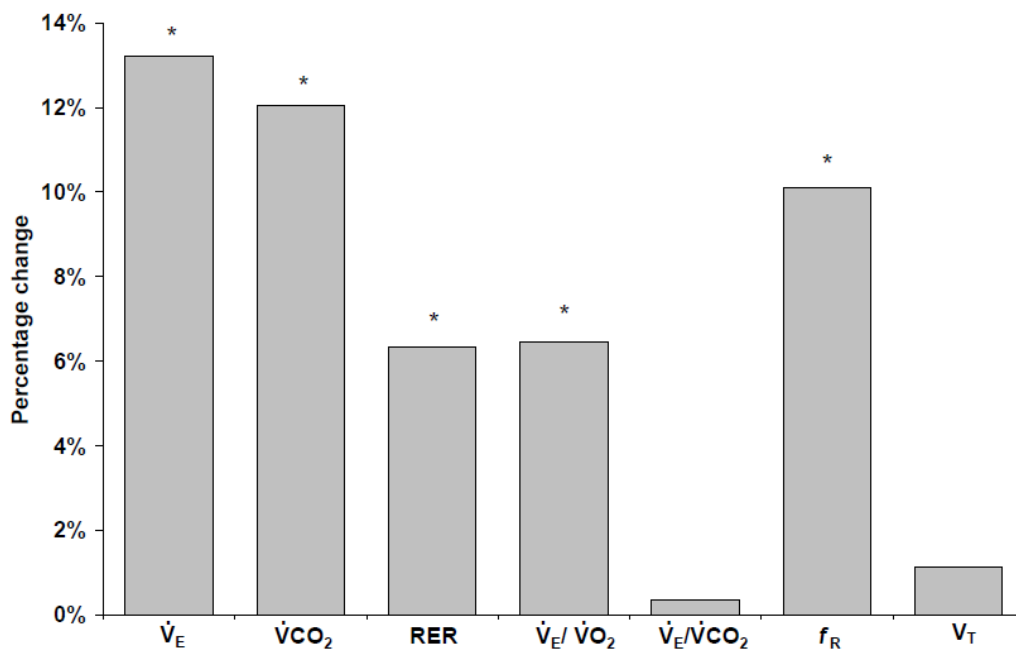


Figure 5.4 Percentage changes in minute ventilation (\dot{V}_E), expired CO_2 ($\dot{V}CO_2$), respiratory exchange ratio (RER), ventilatory equivalent for O_2 ($\dot{V}_E/\dot{V}O_2$), ventilatory equivalent for CO_2 ($\dot{V}_E/\dot{V}CO_2$), breathing frequency (f_R) and tidal volume (V_T) at the $\dot{V}O_2$ value of the pre-eccentric exercise GET. Values are mean (\pm SD).

* Significantly different from pre-eccentric exercise value ($P < 0.05$)

5.5 Discussion

The principal original finding of this investigation is that eccentric, muscle-damaging exercise dissociates the \dot{V}_E and gas exchange responses to ramp incremental exercise from the blood lactate response. While we believe these findings to be novel with respect to exercise-induced muscle damage (EIMD), previous research has demonstrated that the GET and lactate threshold (Tlac) may be dissociated by certain experimental protocols including exercise training (Poole & Gaesser, 1985) and glycogen depletion (Hughes et al., 1982). Thus our findings provide evidence to suggest that following eccentric exercise the control of pulmonary ventilation is influenced by altered or additional stimuli, possibly of neurogenic origin.

As anticipated, the [La] response to ramp incremental cycle exercise was not altered by the EIMD intervention. This finding supports the contention that elevated blood [La] is not an inevitable consequence of EIMD and is, therefore, unlikely to be linked causally to the augmented \dot{V}_E response observed herein and elsewhere (Twist & Eston, 2009) (Chapter 4). Instances of increased blood [La] following eccentric exercise have been linked with a putative switch to more glycolytic energy production possibly as a result of an increased reliance on type II motor unit recruitment (Braun & Dutto, 2003; Chen et al., 2007b; Gleeson et al., 1995, 1998). However, it has been demonstrated that EIMD does not compromise skeletal muscle oxidative function (Walsh et al., 2001) or alter pulmonary $\dot{V}O_2$ kinetics (Schneider et al., 2007).

Our findings contrast with those reported in the only previous study to investigate responses to incremental exercise after eccentric exercise (Gleeson et al., 1998). Gleeson

and co-authors (1998) reported no change in endurance time, $\dot{V}O_2$, \dot{V}_E , or RER responses but found [La] to be higher 48 h after completing a bench-stepping intervention which provoked only mild soreness. Differences in the damage intervention, the incremental protocol employed and the level of muscle soreness induced may account for the disparity in \dot{V}_E and [La] responses between investigations.

There was a small and statistically non-significant difference between the pre-eccentric exercise work rates for the lactate and gas exchange thresholds, with the gas exchange threshold occurring slightly earlier. It should be recognised that the dynamics of blood lactate production and clearance (and hence appearance of lactate in the blood) may differ from the more immediate stimuli for changes in gas exchange and ventilation during ramp incremental exercise. Moreover, gas exchange was measured on a continuous breath-by-breath basis whereas blood samples for [La] determination samples were collected at discrete 1-min intervals. This may increase the potential for disagreement between the lactate and gas exchange thresholds.

The cause and effect relationship between T_{lac} (cause) and GET (effect) is fundamental to the traditional “anaerobic threshold” hypothesis (Beaver et al., 1986; Wasserman et al., 1973, 1990). However, certain experimental conditions have been demonstrated to elicit a dissociation of [La] and \dot{V}_E responses. Under conditions of acute hypoxia (Ozcelik & Kelestimur, 2004) and following prior volitional hyperventilation (Ozcelik et al., 1999) GET is reduced in comparison to the normal, control condition, without a concomitant decrease in T_{lac} . It has been proposed that a ‘pseudo-threshold’ (Whipp, 1987) is evoked by a ‘wash-in’ of CO_2 to the depleted body stores (hyperventilation) or enhanced carotid

body chemosensitivity to CO₂ (hypoxia). However, the reduction in GET observed in the present study does not appear to result from humoral stimuli arising from alterations in blood [La]. Previous investigations reporting a dissociation of \dot{V}_E and [La] responses to exercise have suggested that \dot{V}_E may have been influenced by differences or alterations in the extent of neurogenic control. Patients suffering from McArdle's disease, a condition where a lack of muscle phosphorylase precludes increases in [La] during exercise, demonstrate a dissociation of \dot{V}_E and [La] responses during incremental cycling (Hagberg et al., 1982; Paterson et al., 1990). Similarly, under conditions of reduced muscle glycogen content (Hughes et al., 1982; Sabapathy et al., 2006) and following a period of exercise training (Poole & Gaesser, 1985) \dot{V}_E and [La] responses to exercise are dissociated. However, the existence of a causal link between bicarbonate buffering of lactic acidosis and increases in \dot{V}_E has recently been supported in a study which used DCA to reduce [La] during incremental exercise (Wilkerson et al., 2009). While lactic acidosis may provide an important stimulus to \dot{V}_E it is only one of many factors that can influence the \dot{V}_E response and therefore modulate GET (Dempsey et al., 2006; Ward, 2000; Whipp et al., 1981). It should be mentioned here that while under normal control conditions it is considered that increased non-metabolic CO₂ production from the bicarbonate buffering of lactic acidosis stimulates the increased \dot{V}_E (Wasserman et al., 1973, 1990), in the present study the relative hyperventilation following eccentric exercise might have increased $\dot{V}CO_2$ by 'blowing off' CO₂ from the body stores (Ozcelik et al., 1999) thus reducing the GET as established using the V-slope method (Beaver et al., 1986). Thus we speculate that the augmented \dot{V}_E response and reduced GET observed herein likely result from non-humoral stimuli originating in the damaged, exercising muscle.

The eccentric exercise protocol employed in this study is known to be effective in inducing damage (Byrne & Eston, 2002a, 2002b) (Chapter 4). The primary event which leads to muscle damage from unaccustomed eccentric exercise involves the disruption of sarcomeres and myocyte membranes leading to dysfunction of the excitation-contraction (E-C) mechanism (Proske & Morgan, 2001). As a consequence the immediate and prolonged reductions in peak torque observed in the present investigation may reflect damage to the E-C mechanism. Furthermore the significant increase in plasma CK activity is indicative of increased membrane permeability. While EIMD does not appear to disrupt intrafusal fibres as demonstrated by the unaltered sensitivity of muscle spindle or Golgi tendon afferents (Gregory et al., 2002, 2004), the disruption to extrafusal fibres may provide mechanical stimulation to fine myelinated (group III) and unmyelinated (group IV) afferents (Avela et al., 1999; Komi, 2000; Taguchi et al., 2005). These thin-fibre afferents can exert a significant influence on \dot{V}_E during dynamic exercise (Matiieka & Duffin, 1995) and may have provided an important additional drive to \dot{V}_E leading to the reduction in GET observed in the present study. Similarly, structural and functional disruption to local microvasculature following eccentric exercise (Kano et al., 2005) has been implicated in augmenting the \dot{V}_E response via stimulation of group III and IV afferents (Haouzi et al., 2004). We have previously used an identical eccentric exercise protocol to that employed herein to induce damage and have observed disruption in the matching of O_2 delivery ($\dot{Q}O_2$) to O_2 utilisation ($\dot{V}O_2$), consistent with such microvascular dysfunction, in conjunction with an augmented \dot{V}_E response (Chapter 4) .

Muscular pain is understood to provide an important stimulus to the ventilatory response via the mechanical stimulation of nociceptive muscle afferents (Duranti et al., 1991). Excitation of these afferents via painful electrical muscular stimulation or ischemic muscle pain elicits a reflex increase in breathing frequency (f_R) and minute ventilation (Duranti et al., 1991) whilst concurrently depressing the central neural drive to ventilation (Waldrop et al., 1982). As the mechanical sensitivity of group III and IV thin fibre muscle afferents is augmented by experimental muscle pain as detailed above, so it may be increased by the mechanical hyperalgesia, the sensations of muscle tenderness and movement-induced pain, that result from unaccustomed eccentric exercise. Thus the soreness experienced by our participants during muscle activation may have contributed to the augmented f_R and \dot{V}_E observed following eccentric exercise via afferent neural reflexes.

The elevated ratings of perceived exertion (RPE) reported by our participants corroborate previously reported findings during constant load cycle exercise after eccentric exercise (Twist & Eston, 2009) (Chapter 4) and may account for the reduction in time to exhaustion. This decrease and the associated reduction in WR_{peak} values are consistent with observations of impaired endurance performance brought about by the effects of eccentric exercise (Marcora & Bosio, 2007; Twist & Eston, 2009) (Chapter 4). Submaximal differences in \dot{V}_E , $\dot{V}CO_2$, RER and RPE during ramp exercise are resolved at maximal exercise albeit at a lower work rate and exercise time. This observation supports the proposition that the rating of perceived exertion scales with exercise duration (Crewe et al., 2008; Eston et al., 2007; Faulkner et al., 2008; Joseph et al., 2008) and also that a strong link exists between \dot{V}_E and RPE responses to cycling following eccentric exercise (Chapter 4).

5.6 Conclusion

In conclusion, a prior bout of muscle damaging eccentric exercise augments the \dot{V}_E response to ramp incremental cycling leading to a reduction in GET in the absence of altered blood [La]. The resultant dissociation between GET and T_{lac} indicates that the two phenomena are not causally linked in the presence of EIMD. We propose that the increase in \dot{V}_E and reduction in GET 48 hours after muscle-damaging eccentric exercise are evoked most likely by increased activation of group III and IV afferents stimulated via mechanical disruption of muscle fibres and local microvasculature and are not the result of altered blood [La].

CHAPTER 6

**THE ³¹P-MRS METABOLIC RESPONSE TO INCREMENTAL
EXERCISE FOLLOWING ECCENTRIC,
MUSCLE-DAMAGING EXERCISE**

The contents of this chapter are currently under review for publication:

Davies RC, Eston RG, Fulford J, Rowlands AV and Jones AM. Muscle damage alters the metabolic response to dynamic exercise in humans: a ³¹P-MRS study. *Currently under review.*

6.1 Abstract

This study used ^{31}P -magnetic resonance spectroscopy (^{31}P -MRS) in an attempt to reveal any link between changes in muscle metabolism and the limitations to exercise tolerance in humans with and without exercise-induced muscle damage (EIMD). Ten healthy, physically active men performed incremental knee extensor exercise inside the bore of a whole-body MRS before (pre) and 48 h after (post) performing 100 squats with a load corresponding to 70% of body mass. Time to exhaustion was significantly reduced following EIMD (519 ± 56 and 459 ± 63 s, pre and post EIMD, respectively). End exercise pH (pre: 6.75 ± 0.04 post: 6.83 ± 0.04) and [PCr] (pre: 7.2 ± 1.7 post: 14.5 ± 2.1 mM) values were higher following EIMD (both $P < .05$). However, end exercise [Pi] was not significantly different following EIMD (pre: 19.7 ± 1.9 post: 21.1 ± 2.6 mM, $P > .05$). Resting [Pi] values (pre: 4.7 ± 0.8 post: 6.7 ± 1.7 mM) and consequently [Pi]:[PCr] values (pre: 0.12 ± 0.02 post: 0.18 ± 0.05) were significantly elevated following EIMD and these mean differences were maintained during incremental exercise (all $P < .05$). In contrast, [PCr] and pH values were not different pre and post EIMD at rest or during incremental exercise (all $P > .05$). Findings indicate that alterations in phosphate metabolism, specifically increases in resting [Pi] that are maintained during exercise, may contribute to the reduced exercise tolerance experienced with EIMD.

6.2 Introduction

Unaccustomed eccentrically-biased exercise results in substantial alterations to skeletal muscle structure and function. Morphological changes include disruption of the cytoskeleton, sarcolemma and T-tubules (Fridén and Lieber, 2001), and changes to capillary geometry (Kano et al., 2004). The loss of sarcolemmal integrity results in an efflux of intramyocyte proteins into the bloodstream (Hortobágyi & Denahan, 1989) and an influx of extracellular Ca^{2+} into the sarcoplasm (Armstrong, 1984). These changes are associated with an acute inflammatory response and delayed onset muscle soreness (DOMS) (MacIntyre et al., 1996) and are collectively referred to as exercise-induced muscle damage (EIMD).

Performance decrements associated with EIMD include a reduction in maximal force-generating capacity (Clarkson et al., 1992) and a shorter time to exhaustion (Asp et al., 1998; Carmichael et al., 2005, 2006). Carmichael et al. (2005, 2006) have reported decreases in treadmill run time to fatigue in mice following a bout of downhill running. The decline in performance was associated with increases in the inflammatory cytokine, interleukin 1 β (IL-1 β) within regions of the brain responsible for movement, motivation, perception of effort and pain (Carmichael et al., 2005). Central fatigue factors such as the enhanced production of inflammatory cytokines have also been implicated in reduced time trial running (Marcora and Bosio, 2007) and cycling performances in humans (Twist and Eston, 2009,) where an elevated perception of exertion appeared to mediate performance following eccentric exercise. Peripheral fatigue factors originating within the damaged muscle tissue may also be involved in the reduced time to exhaustion following eccentric, muscle-damaging exercise. Using muscle biopsy procedures, Asp et al. (1998) reported

decreases in muscle glycogen content in human subjects following muscle-damaging exercise which were associated with a 23% reduction in maximal work capacity during incremental knee extensor exercise. Other observed changes in metabolic function following eccentric exercise include impaired muscle glycogen resynthesis (Asp et al., 1995, 1998) and an elevated blood lactate response during exercise (Gleeson et al., 1995, 1998; Asp et al., 1998). It is feasible that these changes reflect a shift in the muscle metabolic profile to an increased reliance on non-oxidative metabolism, contributing to the decreased endurance capacity following EIMD (Asp et al., 1998). However, the precise nature of the accelerated fatigue development experienced with EIMD is poorly understood.

Non-invasive evaluation of muscle metabolism can be achieved using ^{31}P -magnetic resonance spectroscopy (^{31}P -MRS). Several studies have used this technology to demonstrate an increase in the resting inorganic phosphate (Pi) to phosphocreatine (PCr) ratio following EIMD, suggestive of an increase in resting muscle metabolism (McCully 1992, Rodenburg et al., 1995; Lund et al., 1998a, 1998b). Several of the candidate mechanisms for the accelerated development of fatigue and reductions in peak power observed following eccentric exercise, including increases in Pi and ADP and decreases in pH, can be measured using ^{31}P -MRS. Rodenburg et al. (1995) reported no difference in the Pi:PCr ratio, pH or peak power during graded knee extensor exercise performed 24 h after a bout of stepping exercise designed to induce EIMD. However, the authors concluded that the lack of change in several markers of muscle damage indicated that the muscle-damaging protocol employed was not sufficiently severe to alter muscle metabolism (Rodenburg et al., 1995).

It has been demonstrated that an effective muscle-damaging protocol reduces time to exhaustion during high-intensity cycling (Chapter 4). However the mechanisms underlying the reduced endurance capacity following muscle-damaging exercise remain to be determined. Central mechanisms which may potentially influence the increased development of fatigue, such as increased inflammatory cytokine production are difficult to evaluate during dynamic exercise in the human model. Putative peripheral fatigue mechanisms including an increased rate of [PCr] depletion, an increased accumulation of [Pi] and [ADP] and a decreased rate of fall in pH can all be assessed using ^{31}P -MRS. Following a period of endurance training, incremental knee extensor exercise tolerance is enhanced with the relationships of [Pi]:[PCr], [PCr] and pH against time demonstrating a rightward shift (Jones et al., 2007). It is possible that following a bout of muscle-damaging exercise the reduced exercise tolerance observed could be associated with a leftward shift in these ^{31}P metabolite-time profiles. Alternatively, an unchanged muscle metabolite response would indicate that other, potentially central mechanisms may be responsible for any decrements in endurance capacity. The purpose of this study was therefore to investigate the effect of a well-defined bout of eccentric, muscle-damaging exercise (Chapter 3) on changes in muscle metabolism during dynamic incremental knee extensor exercise. We used ^{31}P -MRS to test the hypothesis that exercise-induced muscle damage (EIMD) alters the muscle metabolic response to dynamic exercise and thus limits exercise tolerance in humans.

6.3 Methods

Subjects

Ten healthy, physically active male subjects (age, 22 ± 4 years; mass, 78.2 ± 8.8 kg; height, 1.79 ± 0.08 m) volunteered to participate in this study. All participants were asymptomatic of illness and pre-existing injuries and had not performed any resistance training of the lower limbs within the previous six months. Participants provided written informed consent to participate in the study which was approved by the Institutional Ethics Committee (See appendices B, C and D for exemplar participant information sheet, participant consent form and ethical approval certificate).

Assessment of muscle damage

All indicators of muscle damage, perceived muscle soreness (using a 0-10 visual analogue scale (VAS)), creatine kinase (CK) activity and isokinetic peak torque ($30 \text{ deg}\cdot\text{s}^{-1}$), were measured in the order listed, immediately before, and 24 and 48 h after performing the eccentric, muscle-damaging exercise protocol. For further details of these procedures please refer to Chapter 3.

Experimental procedures

Following the assessment of muscle damage, single -legged, knee-extension exercise tests were completed at the same time of day ± 1 h for each participant, before and 48 h after performing eccentric, muscle-damaging exercise. The dynamic knee-extensor exercise tests were conducted in the prone position with the subjects positioned inside a whole body MRI system. A 6-cm ^{31}P transmit-receive surface coil was placed within the subject bed, and the subject was asked to lie on it such that the coil was centred over the quadriceps

muscle of the leg to be exercised. Subjects were then secured to the ergometer bed with Velcro straps at the thigh, buttocks, and lower back to minimise extraneous movement during the protocol. The foot of the leg to be exercised was connected to a pulley system that permitted a nonmagnetic weight to be lifted and lowered and work rate to be calculated (Figure 6.1).

Exercise was performed at a rate of 40 repetitions/min with the subjects lifting and lowering the mass over a distance of ~0.22 m in accordance with a visual cue projected onto the front wall of the scanner room. The contraction phase of the knee extensors and the ³¹P-MRS interrogation of the quadriceps occurred in unison. After a 2-min period of rest, the subjects commenced knee-extension exercise against an initial basket load of 1 kg. Thereafter, the basket load was increased by 0.5 kg every 30 s until the subjects were no longer able to maintain the kicking frequency at 40 repetitions/min. The subjects received strong verbal encouragement to continue for as long as possible while maintaining appropriate form.

Eccentric, muscle-damaging exercise protocol

Participants completed 100 (Smith) squats, performed as 10 sets of 10 repetitions with the load on the bar corresponding to ~70% of each participant's body mass. Prior to commencing, all participants were instructed in correct and safe lifting technique. The bar was positioned on the participant's shoulders and feet were positioned under the bar, with the back straight and legs fully extended (knee = 180°). The descent phase involved eccentric action of the knee extensors to lower the bar to a knee angle of just past 90°. The

lifting phase involved concentric action to return the bar to the starting position. For further details of these procedures please refer to Chapter 3.

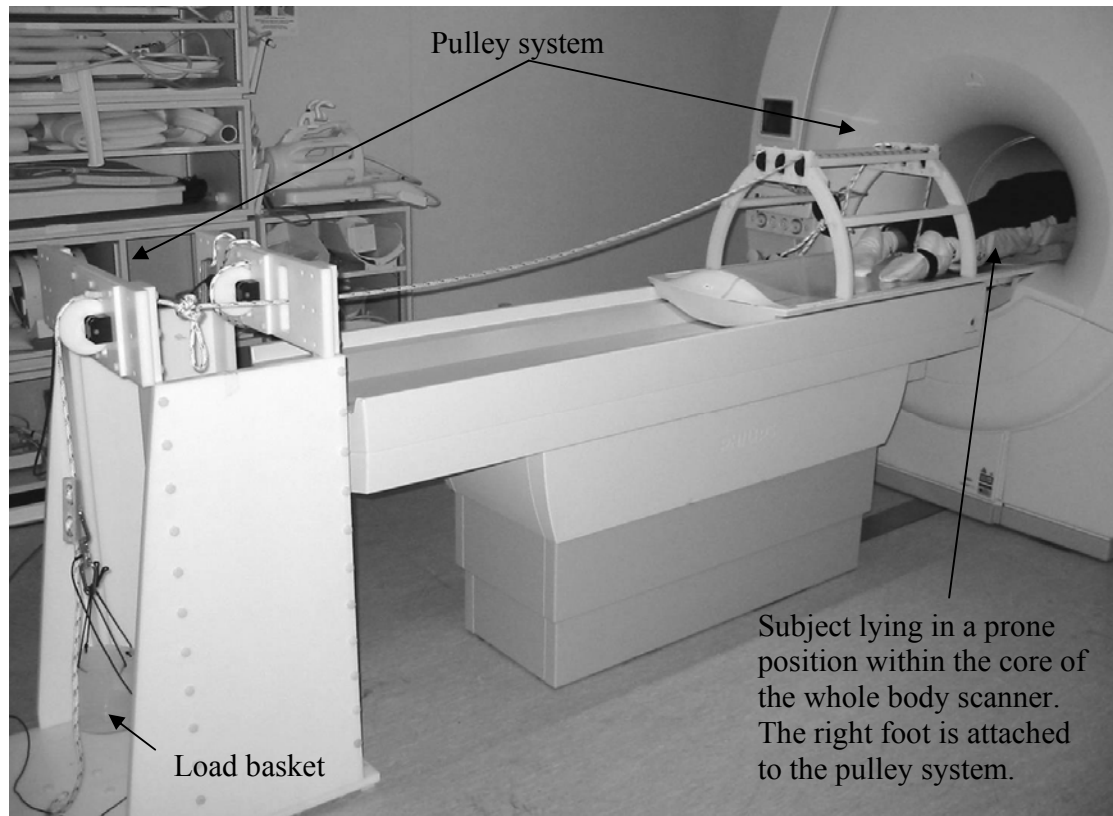


Figure 6.1 The MRS knee extensor ergometer showing pulley system and load basket.

Measurements

MRS measurements

MRS was performed in the Peninsula Magnetic Resonance Research Centre using a 1.5-T superconducting magnetic resonance scanner (Philips Gyroscan Clinical Intera, Philips Medical Systems, Best, Netherlands). Initially, fast-field echo images were acquired to

determine whether the muscle was positioned correctly relative to the coil. This was aided by placing cod liver oil capsules, which yield high-intensity signal points within the image, adjacent to the coil, allowing its orientation relative to the muscle volume under examination to be assessed. A number of preacquisition steps were carried out to optimise the signal from the muscle under investigation. Tuning and matching of the coil were then performed, followed by an automatic shimming protocol undertaken within a volume that defined the quadriceps muscle. The muscle volume from which signal originates is dictated by the sensitive volume of the coil itself. This approximates to the physical size of the coil and thus corresponds to a cylinder of 6cm diameter and 6cm deep adjacent to the coil. Outside of this volume, muscle will still contribute to the signal but to an extent that drops off rapidly as you move away from the sensitive region. To ensure that the examined muscle was consistently at the same point relative to the coil during exercise, the subject was visually queued via a display consisting of two vertical bars, one that moved at a constant rate with a frequency of 0.67 Hz and one that monitored foot movements via a sensor present within the pulley to which they were connected. Thus the subject endeavoured to match the movements of these two bars. The work done by the subjects was recorded via a nonmagnetic strain gauge present within the pulley mechanism. Before exercise, during exercise, and during recovery, data were acquired every 1.5 s, with a spectral width of 1,500 Hz and 1,000 data points. Phase cycling with eight phase cycles was employed, leading to a spectra being acquired every 12 s. The subsequent spectra were quantified via peak fitting, assuming prior knowledge, using the jMRUI (version 2) software package and the AMARES fitting algorithm (Vanhamme et al., 1997; Naressi et al., 2001).

Spectra were fitted assuming the presence of the following peaks: Pi, phosphodiester, phosphocreatine (PCr), α -ATP (2 peaks, amplitude ratio 1:1), γ -ATP (2 peaks, amplitude ratio 1:1), and β -ATP (3 peaks, amplitude ratio 1:2:1). In all cases, relative amplitudes were corrected for partial saturation because of the short repetition time relative to the longitudinal relaxation time constant T1. Absolute concentrations were determined by calculating the size of peak areas relative to β -ATP which was set at 8.2 mM. The ratio of Pi to PCr was determined from the respective Pi and PCr spectral areas as obtained during the quantification procedure. Intracellular pH was calculated from the chemical shift of the Pi spectral peak relative to the PCr (Moon and Richards, 1973). ADP concentrations were calculated as described by Kemp et al., (2001) taking into account the pH dependency of the binding of H⁺, K⁺ and Mg²⁺. To determine the intracellular threshold (IT) during incremental exercise, piecewise linear regression was used. Briefly, different two-line combinations were fitted to the [Pi]/[PCr]-work rate and pH-work rate relationships until the lowest sum of squared residuals was found (Hogan et al., 1983; Marsh et al., 1991). The point at which this particular two-line combination intersected was accepted as the IT.

Statistical analysis

Changes in the indicators of muscle damage (perceived muscle soreness, creatine kinase activity and isokinetic peak torque) were analysed using a series of one-way repeated measures ANOVAs. As the CK activity data were not normally distributed these values were log-transformed prior to statistical analysis (Twist & Eston, 2005) (see appendix H).

Paired *t*-tests were used to determine significant differences in end exercise values between the two conditions. Where data were not normally distributed Wilcoxon tests were also run. Changes in the MRS measurements (Pi, PCr, Pi:PCr ratio and pH) at rest and

after 2, 4 and 6 min of incremental exercise were examined using separate two-way fully repeated measure ANOVAs (condition x time). The assumption of sphericity was evaluated using Mauchly's test. Where sphericity was violated ($P < 0.05$), the Greenhouse-Geisser (GG) correction factor was applied. All data were analysed using the statistical software package SPSS for Windows (version 13) with statistical significance set at 0.05. Values are means \pm SD.

6.4 Results

Muscle damage

There were significant changes in all indicators of muscle damage following eccentric exercise. Table 6.1 shows changes in muscle soreness, CK activity and isokinetic peak torque before and after eccentric exercise. Muscle soreness increased 24 h after eccentric exercise with the highest values reported at 48 h ($F_{(2, 18)} = 26.22, P < 0.05$). Plasma CK activity increased after eccentric exercise, with the highest values observed at 24 h ($F_{GG(1.1, 9.9)} = 15.02, P < 0.05$). Isokinetic peak torque ($30 \text{ deg}\cdot\text{s}^{-1}$) decreased by 15% at 24 h and remained 11% lower than baseline values at 48 h ($F_{(2, 18)} = 14.33, P < 0.05$).

Table 6.1 Changes in indicators of muscle damage. Mean \pm SD values and (range) before and 24 h and 48 h after eccentric exercise.

Measured Variable	before	24 h	48 h
Soreness (0-10 VAS)	0.5 \pm 0.3 (0.1 – 1.1)	5.0 \pm 2.0* (1.8 – 8.2)	5.5 \pm 3.2* (1.6 – 9.7)
CK activity (U/L)	178 \pm 61 (97 – 270)	798 \pm 692* (196 – 2481)	409 \pm 264* (213 – 1088)
Peak Torque (N.m) 30 deg.s⁻¹	310 \pm 47 (227 – 366)	265 \pm 66* (113 – 340)	275 \pm 77* (115 – 361)

Soreness, visual analogue scale (VAS) 0–10. CK, creatine kinase.

* Significantly different from pre-eccentric exercise value ($P < 0.05$).

MRS measurements

As anticipated, time to exhaustion (519 ± 56 and 459 ± 63 s, pre and post muscle damage respectively) and associated peak work rate values (29 ± 4 and 25 ± 4 W, pre and post muscle damage respectively) attained during the incremental knee extensor exercise were significantly reduced following muscle damage ($t_{(9)} = 4.85$, $P < 0.05$ and $t_{(9)} = 5.21$, $P < 0.05$, respectively). End exercise values are presented in Table 6.2. The data for end exercise [Pi]:[PCr] and ADP were not normally distributed therefore Wilcoxon tests were used to analyse these data. However results did not differ from t -test results therefore for consistency the results of the paired t -tests are presented.

Table 6.2 Muscle metabolic responses at rest and during incremental exercise before (Pre) and 48h after (Post) eccentric, muscle-damaging exercise. Values are means \pm SD.

Measured Variable		Resting	2 min	4 min	6 min	End
[PCr] (mM) [†]	Pre	38.3 \pm 1.2	35.0 \pm 2.1	30.7 \pm 3.1	24.3 \pm 4.0	7.2 \pm 1.7
	Post	37.9 \pm 1.4	34.1 \pm 2.6	31.3 \pm 3.0	25.5 \pm 4.8	14.5 \pm 2.1*
[Pi] (mM) [†]	Pre	4.7 \pm 0.8	5.6 \pm 1.1	8.0 \pm 1.9	10.6 \pm 3.0	19.7 \pm 1.9
	Post [‡]	6.7 \pm 1.7	7.5 \pm 2.1	10.4 \pm 4.0	14.8 \pm 6.5	21.1 \pm 2.6
[Pi]:[PCr] ratio [†]	Pre	0.12 \pm 0.02	0.16 \pm 0.03	0.26 \pm 0.05	0.45 \pm 0.13	2.09 (1.74-14.4) ^{††}
	Post [‡]	0.18 \pm 0.05	0.22 \pm 0.07	0.34 \pm 0.15	0.62 \pm 0.34	1.46 (0.85-2.29) ^{††*}
pH [†]	Pre	7.02 \pm 0.03	7.06 \pm 0.04	7.03 \pm 0.04	6.96 \pm 0.06	6.75 \pm 0.04
	Post	7.02 \pm 0.03	7.07 \pm 0.03	7.04 \pm 0.05	6.99 \pm 0.09	6.83 \pm 0.04*

[†] Significant main effect for time (resting, 2, 4 and 6 min) ($P < 0.05$)

[‡] Significant main effect for condition (Pre and Post) ($P < 0.05$)

* Significantly different from Pre-eccentric exercise ($P < 0.05$)

^{††} End exercise [Pi]:[PCr] ratio data were not normally distributed therefore values are presented as median (interquartile range).

Table 6.3 Intracellular Threshold (IT) values before (Pre) and 48h after (Post) eccentric, muscle-damaging exercise. Values are means \pm SD.

Measured Variable	Pre	Post
Time @ IT (s)	326 \pm 77	316 \pm 65
[Pi]:[PCr] ratio @ IT	0.30 \pm 0.14	0.31 \pm 0.10
pH @ IT	7.07 \pm 0.02	7.08 \pm 0.02

End exercise pH and [PCr] values were higher ($t_{(9)} = -2.34$, $P < 0.05$ and $t_{(9)} = -4.49$, $P < 0.05$, respectively) and end exercise [Pi]:[PCr] values were lower ($t_{(9)} = 2.346$, $P < 0.05$,) following eccentric exercise. However, end exercise [Pi] and ADP were not significantly different following eccentric exercise ($t_{(9)} = -0.496$, $P > 0.05$ and $t_{(9)} = 1.82$, $P > 0.05$, respectively). The IT was not significantly altered by eccentric exercise ($t_{(9)} = 0.51$, $P > 0.05$). The time, [Pi]:[PCr] and pH values when the IT occurred are presented in Table 6.3.

Prior to eccentric exercise, all ten participants completed a minimum of 7 min exhaustive incremental exercise. However, 48 h after eccentric exercise the minimum time achieved by all participants was reduced to 6 min. Thus, the first 6 min of incremental exercise were analysed in order to examine changes in muscle metabolic responses during exercise.

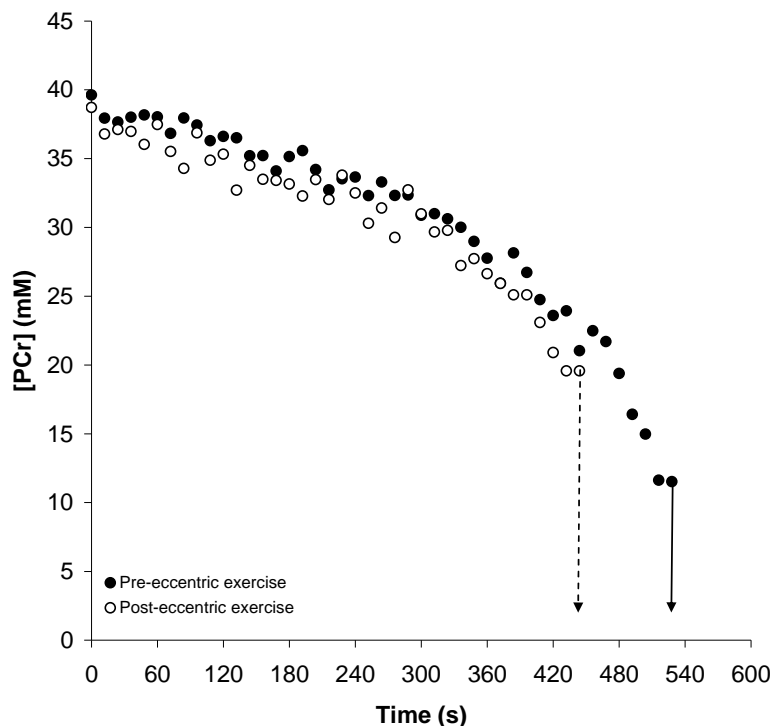


Figure 6.2 [PCr] response of a representative participant, pre- and post-eccentric, muscle damaging exercise. Vertical arrows indicate time to exhaustion in the two exercise conditions. The solid line represents time to exhaustion pre-eccentric exercise; the dashed line represents time to exhaustion post-eccentric exercise.

Muscle metabolic responses at rest and during incremental exercise are presented in Table 6.2 (see appendix I for graphical representation of these data). There was a significant main effect for time for all muscle metabolic responses, with [PCr] and pH values declining, and [Pi] and [Pi]:[PCr] values increasing as the incremental exercise progressed (all $P < 0.05$). There was a significant main effect for condition for [Pi] ($F_{(1,9)} = 7.080$, $P < 0.05$). Resting [Pi] values were significantly elevated and this mean difference was maintained during the first 6 min of incremental exercise.

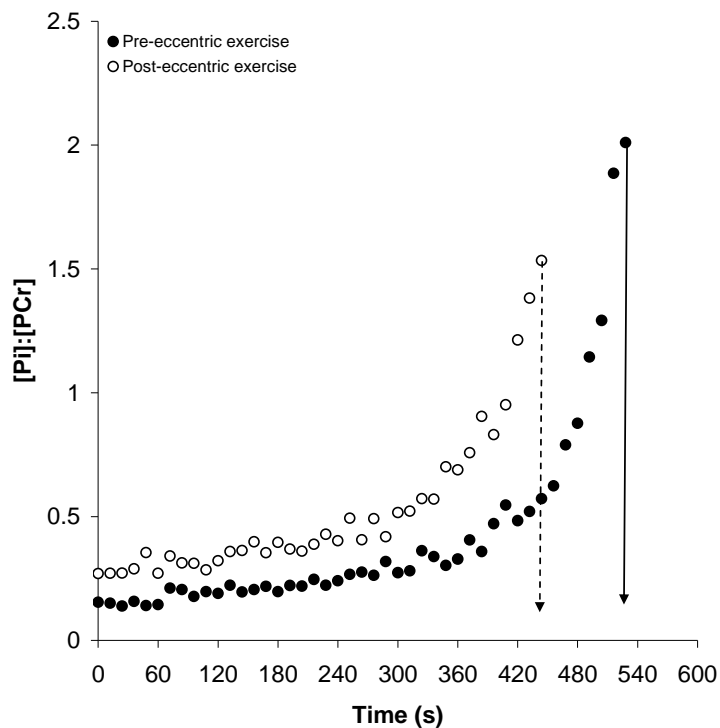


Figure 6.3 [Pi]:[PCr] response of a representative participant, pre- and post-eccentric, muscle damaging exercise. Vertical arrows indicate time to exhaustion in the two exercise conditions. The solid line represents time to exhaustion pre-eccentric exercise; the dashed line represents time to exhaustion post-eccentric exercise.

As a direct consequence, $[Pi]:[PCr]$ values were also elevated at rest and during the first 6 min of exercise ($F_{(1,9)} = 5.908, P < 0.05$). In contrast, $[PCr]$ and pH values were not different between conditions at rest and during incremental exercise (both $P > 0.05$). There were no significant interactions of condition and time for any muscle metabolic responses. Changes in $[PCr]$, $[Pi]:[PCr]$ and pH are illustrated for a typical participant in Figures 6.2 - 6.4.

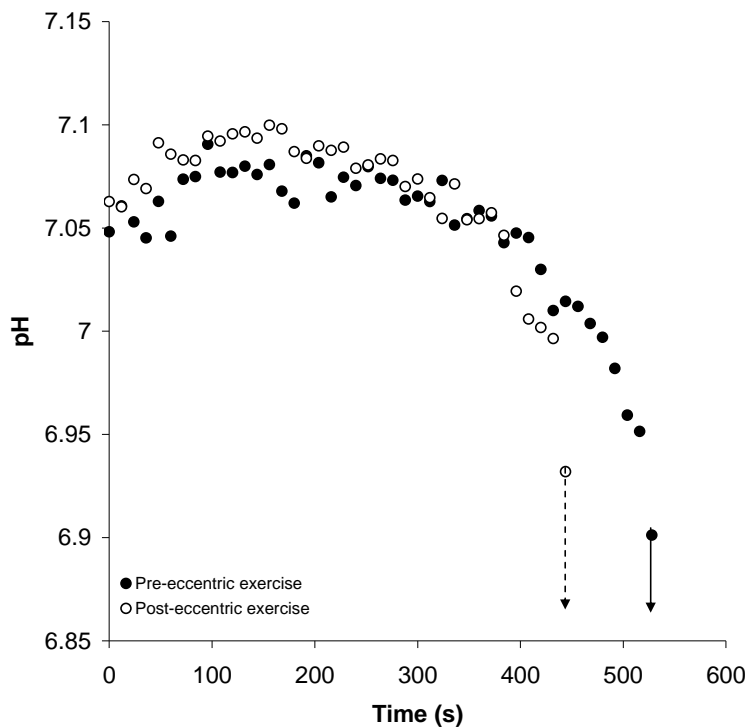


Figure 6.4 pH response of a representative participant, pre- and post-eccentric, muscle damaging exercise. Vertical arrows indicate time to exhaustion in the two exercise conditions. The solid line represents time to exhaustion pre-eccentric exercise; the dashed line represents time to exhaustion post-eccentric exercise.

6.5 Discussion

To our knowledge, this is the first study to investigate changes in muscle metabolism with ^{31}P -MRS during incremental knee extensor exercise following an effective muscle-damaging protocol. The principal original finding of this investigation was that the reduction in time-to-exhaustion consequent to a bout of eccentric, muscle-damaging exercise was not associated with an accelerated depletion of [PCr] or faster fall in pH. Specifically, time-to-exhaustion was reduced by 12% following the muscle-damaging exercise but the temporal changes in [PCr] and pH were similar in the two experimental conditions such that end-exercise [PCr] and pH were significantly higher 48 h after the muscle-damaging exercise (Figures 6.1 and 6.3). In contrast, the end-exercise [Pi] was not significantly different between the pre- and post-EIMD conditions. These results indicate that the reduced exercise tolerance following EIMD may be related either to the increased [Pi] that was observed at rest and throughout incremental exercise, or to other unmeasured peripheral or central factors. Importantly, however, the results allow us to discount a greater rate of non-oxidative energy metabolism (as inferred from the changes in [PCr] and pH) as important mediators of the reduced exercise tolerance following EIMD.

Our findings provide novel insights into changes in ^{31}P metabolite responses to dynamic exercise subsequent to muscle-damaging exercise. Previous research has reported unaltered Pi:PCr ratio, pH and peak power during incremental exercise following a prior bout of eccentric exercise (Rodenburg et al. 1995). However, these authors speculated that the bench-stepping protocol employed to induce damage was not sufficiently strenuous to elicit changes in exercise metabolism. Indeed, the lack of change in several markers of muscle damage including maximum power output suggests that their eccentric exercise

protocol was ineffective (Rodenburg et al. 1995). Participants in the present study completed a bout of eccentric, muscle-damaging exercise, comprising 100 squats with the load on the bar set to 70% body mass (see chapter 3). This eccentric exercise protocol is known to be effective in inducing damage (Byrne and Eston 2002a, 2002b). Indeed, in the present study, there were changes in all measured symptoms of muscle damage. The disruption of sarcomeres and myocyte membranes during unaccustomed eccentric exercise leads to immediate and prolonged reductions in peak torque due to dysfunction of the excitation-contraction (E-C) mechanism (Proske and Morgan 2001). The 11% reduction in isokinetic peak torque observed at 48 h is consistent with previous studies which have employed this eccentric exercise protocol in reflecting damage to the E-C mechanism (Byrne and Eston 2002a, 2002b). Furthermore the significant increase in plasma CK activity is indicative of increased membrane permeability. Impaired maximal force production may, in part, account for the reduction in time to exhaustion and the associated 14% reduction in peak work rate values. The accelerated rate of fatigue experienced by our participants is consistent with observations of impaired endurance performance brought about by the effects of eccentric exercise (Asp et al. 1998; Marcora and Bosio 2007; Twist and Eston 2009).

Peripheral fatigue factors originating from within the damaged muscle tissue have been implicated in the reduction in endurance capacity associated with exercise-induced muscle damage. Specifically, reports of increased blood lactate (Asp et al., 1998; Braun & Dutto, 2003; Chen et al., 2007) and increased utilisation of glycogen stores (Asp et al., 1998) following eccentric exercise have been attributed to a putative shift towards an increased reliance on anaerobic energy production leading to impaired endurance performance (Asp

et al., 1998; Braun & Dutto, 2003; Chen et al., 2007). However, in the present study, the PCr response for a given work rate was not significantly altered, although the performance of incremental knee extensor exercise was impaired. It is recognised that muscle PCr and $\dot{V}O_2$ demonstrate similar kinetic profiles during transitions to higher exercise intensities (Barstow et al., 1994; Mahler, 1985; Marsh et al., 1993), indicating that the rate of oxidative phosphorylation is closely linked to PCr hydrolysis (Mahler, 1985, Meyer, 1988). Thus our observation of an unchanged PCr profile during incremental exercise is consistent with reports of unchanged $\dot{V}O_2$ kinetics following eccentric exercise (Schneider et al., 2007). Collectively these data indicate that exercise-induced muscle damage does not compromise oxidative function during dynamic exercise. The reduction in time to fatigue resulted in significant alterations in end exercise [PCr] values. Prior to the bout of muscle-damaging exercise the mean depletion of the PCr pool was over 80% at the end of the incremental exercise, with several subjects almost completely depleting their muscle PCr. However 48 h after the muscle-damage was induced, participants reached exhaustion sooner with a mean depletion of the PCr pool of approximately 62%. Thus end-exercise [PCr] was higher when incremental exercise was performed in the muscle-damaged condition.

Similarly, intracellular pH demonstrated an unchanged rate of fall during incremental exercise after muscle-damage was induced. There was no significant difference in the pH values for a given work rate at 48 h although end exercise pH was higher due to the shorter time to exhaustion. Furthermore, the unchanged [PCr] and pH responses to incremental exercise resulted in there being no significant difference in the IT values following eccentric exercise. Low [PCr] and pH have been implicated in the fatigue process

(Westerblad and Allen 2003; Wilson et al. 1988). However, on the evidence of our observations these effects do not appear to be associated with the reduction in exercise tolerance that accompanies exercise-induced muscle damage.

At rest, increases in [Pi], resulted in a 50% increase in the resting [Pi]:[PCr] ratio 48 h after eccentric exercise. These findings are consistent with those of several previous studies using ³¹P-MRS in reporting increases in resting [Pi]:[PCr] following eccentric exercise (McCully 1992, Rodenburg et al., 1995; Lund et al., 1998a, 1998b). Elevated resting [Pi] such as observed herein and elsewhere (McCully 1992, Rodenburg et al., 1995; Lund et al., 1998a, 1998b) may be interpreted as an increase in muscle metabolism. The repair and remodelling of tissue damaged via eccentric exercise such as that reported by Yu et al. (2002) and Yu and Thornell (2002) could lead to an increase in resting muscle metabolism. Furthermore, depleted resting muscle glycogen content following eccentric exercise, particularly the content of the preferentially damaged type II fibres, has been attributed to increased resting muscle glycogen utilisation (Asp et al., 1998). An alternative explanation for the increase in [Pi]:[PCr] which, importantly, results from increased [Pi] but not decreased [PCr], may be linked to the breakdown of muscle tissue consequent to eccentric exercise. Disturbances in intracellular Ca²⁺ following eccentric exercise have been reported with concomitant activation of calcium-activated proteolytic pathways (Belcastro, 1993; Belcastro et al. 1998). Thus the observed increase in resting [Pi] may not be due to increases in muscle metabolism but to the degradation of muscle proteins.

Increases in resting [Pi] and [Pi]:[PCr] following a period of cast immobilisation have been implicated in the loss of muscular strength resultant to the period of disuse (Pathare et al.,

2005, 2008). Similarly, increases in the resting [Pi]:[PCr] ratio of patients with postpolio residual paralysis, a condition which is characterised by decreased endurance capacity and muscular weakness, is related to the severity of paralysis (Sharma et al., 2007). Increases in intracellular [Pi] can inhibit force production via direct action on cross-bridge formation or on sites in the excitation-contraction pathway and may play a key role in the development of muscle fatigue (Westerblad et al., 2002). Thus the reduction in peak torque and exercise tolerance observed in the present study following muscle damage, may be related to the increased [Pi] and [Pi]:[PCr] which was observed not only at rest but also during incremental exercise. The novel observation that the significant increases in resting [Pi] and [Pi]:[PCr] at 48 h were maintained during the dynamic exercise test, is of particular interest. The rate of increase in [Pi] during incremental exercise was not altered as a result of the muscle damage. However the premature termination of the test at 48 h resulted in there being no significant difference in end exercise [Pi] values. It might be tempting to speculate that the limit to exercise tolerance was moderated by [Pi]. However wide inter-subject variability was observed with the [Pi] values, thus we cannot reliably conclude that [Pi] is indeed a limiting factor. Rather that end exercise [Pi] most likely makes an important contribution to the reduced time to exhaustion experienced following muscle damaging exercise.

Central fatigue factors including the production of inflammatory cytokines may be involved in the reduced time to exhaustion following eccentric exercise observed herein. Carmichael et al. (2005) have reported increases in brain IL-1 β in areas responsible for movement, motivation, perception of effort and pain, which have been associated with decreases in treadmill runs to fatigue in mice. In human subjects, it has been proposed that the increased

sense of effort reported during dynamic exercise mediates time trial performance following eccentric exercise (Marcora & Bosio 2007; Twist & Eston 2009). The duration of an individual's incremental exercise to 'volitional exhaustion' is regulated by a complex interaction of central and peripheral fatigue factors. However, the decision to terminate exercise is ultimately a conscious behavior based on the perception of alterations in sub-conscious homeostatic control systems (St Clair Gibson et al., 2003). It is beyond the scope of this study to determine whether central or peripheral factors make the greater contribution to the accelerated fatigue development experienced with EIMD.

6.6 Conclusion

In conclusion, the results of this study suggest that the reduced exercise tolerance following EIMD cannot be attributed to a greater rate of non-oxidative energy metabolism (as inferred from the changes in [PCr] and pH). Although we cannot exclude an important role for centrally-mediated fatigue, our results indicate that increases in resting [Pi], that are maintained during exercise, may be a contributory factor to the reduced exercise tolerance that is observed following EIMD.

CHAPTER 7

THE EFFECT OF ECCENTRIC EXERCISE-INDUCED MUSCLE DAMAGE ON THE DYNAMICS OF MUSCLE OXYGENATION AND PULMONARY OXYGEN UPTAKE

The contents of this chapter form the basis of the following publication /presentation:

Publication

Davies RC, Eston RG, Poole DC, Rowlands AV, Dimenna F, Wilkerson DP, Twist C, and Jones AM. The effect of eccentric exercise-induced muscle damage on the dynamics of muscle oxygenation and pulmonary oxygen uptake. *J Appl Physiol* (August 14, 2008). doi:10.1152/jappphysiol.90743.2008

Presentation

Davies R, Eston R, Poole D, Rowlands A, Dimenna F, Wilkerson D, Twist C, and Jones A. The influence of exercise-induced muscle damage on pulmonary oxygen uptake and muscle deoxygenation kinetics during high intensity exercise. *13th Annual Congress of the European Congress of Sports Sciences, Book of Abstracts*, edited by Cabri J, Alves F, Araujo D, Barreiros J and Veloso A, Estoril, 2008, p630.

7.1 Abstract

Unaccustomed eccentric exercise has a profound impact on muscle structure and function. However, it is not known whether associated microvascular dysfunction disrupts the matching of O_2 delivery ($\dot{Q}O_2$) to O_2 utilisation ($\dot{V}O_2$). Near infra-red spectroscopy (NIRS) was used to test the hypothesis that eccentric exercise-induced muscle damage would elevate the muscle $\dot{Q}O_2:\dot{V}O_2$ ratio during severe intensity exercise whilst preserving the speed of the $\dot{V}O_2$ kinetics at exercise onset. Nine physically active men completed 'step' tests to severe-intensity exercise from an unloaded baseline on a cycle ergometer before and 48 h after eccentric exercise (100 squats with a load corresponding to 70% of body mass). NIRS and breath-by-breath pulmonary $\dot{V}O_2$ were measured continuously during the exercise tests and subsequently modelled using standard non-linear regression techniques. There were no changes in phase II pulmonary $\dot{V}O_2$ kinetics following the onset of exercise (time constant, pre: 25 ± 4 ; post: 24 ± 2 s; amplitude, pre: 2.36 ± 0.23 ; post: 2.37 ± 0.23 L/min; all $P > 0.05$). However, the primary (pre: 14 ± 3 ; post: 19 ± 3 s) and overall (pre: 16 ± 4 ; post: 21 ± 4 s) mean response time of the [HHb] response was significantly slower following eccentric exercise ($P < 0.05$). The slower [HHb] kinetics observed following eccentric exercise is consistent with an increased $\dot{Q}O_2:\dot{V}O_2$ ratio during transitions to severe-intensity exercise. We propose that unchanged primary phase $\dot{V}O_2$ kinetics are associated with an elevated $\dot{Q}O_2:\dot{V}O_2$ ratio that preserves blood-myocyte O_2 flux.

7.2 Introduction

Unaccustomed eccentric exercise has a profound impact on muscle structure and function. Following such exercise, myocytes demonstrate ultra-structural changes including sarcomere disruption described as ‘popping’ (Morgan, 1990), ‘Z-band streaming’ (Fridén & Lieber, 2001; Stupka et al., 2001) or ‘smearing’ (Kano et al., 2005), and damage to t-tubules, sarcoplasmic reticulum and sarcolemma (Fridén & Lieber, 2001). This disruption leads to increased influx of extracellular Ca^{2+} into the sarcoplasm, leading to enhanced proteolytic enzyme activity (Proske et al., 2004) and an accompanying inflammatory response (Fielding et al., 1993). In addition, intramyocyte contents such as creatine kinase and myoglobin are released into the bloodstream (Warren et al., 1999). These degenerative changes are associated with delayed onset muscle soreness (DOMS) and a reduction in maximal force generating capacity (Byrne et al., 2004; Clarkson, 1992; Cleak & Eston, 1992).

As myocyte degeneration is known to lead to decrements in maximal force production, any associated damage to the microcirculation could potentially have an adverse effect on sub-maximal locomotory activity such as running or cycling. Activities that require repetitive low-force contractions rely on effective vascular function that ensures an adequate blood and O_2 supply to muscle. Accordingly, Kano et al. (2005) have reported substantial microvascular dysfunction in rat spinotrapezius muscle following unaccustomed eccentric exercise (downhill running). Specifically, these authors reported an increase in the proportion of capillaries that did not support red blood cell (RBC) flux and an increase in mean capillary diameter in resting muscle. Furthermore, an accelerated fall in microvascular oxygen pressure was observed at the onset of electrically stimulated

contractions. Microcirculatory dysfunction such as this could conceivably lead to impaired delivery and distribution of O₂ within the capillary bed. Similarly, the matching of O₂ delivery ($\dot{Q}O_2$) and O₂ utilisation ($\dot{V}O_2$) at the onset of exercise might be disturbed, thereby compromising blood-muscle O₂ flux and, if sufficiently severe, slow the kinetic adaptation of $\dot{V}O_2$ at exercise onset (Kano et al., 2005). Although the compelling weight of evidence supports the premise that $\dot{V}O_2$ kinetics in healthy individuals are not limited by O₂ delivery, *per se*, in disease conditions such as chronic heart failure (Richardson et al., 2003) and Type II diabetes (Padilla et al., 2006) where vascular function and capillary haemodynamics are impaired, $\dot{V}O_2$ kinetics are slowed (Poole et al., 2005). It is possible that microcirculatory dysfunction brought about by previous eccentric exercise could result in the $\dot{V}O_2$ kinetics of healthy individuals becoming slower due to a muscle O₂ delivery limitation. That is, the muscle damage could cause individuals to cross the so-called ‘tipping point’ beyond which reductions in muscle O₂ availability begin to measurably lengthen the time constant describing the phase II $\dot{V}O_2$ response (Poole et al., 2008).

Near infrared spectroscopy (NIRS) facilitates the assessment of muscle (haemoglobin + myoglobin) oxygenation and can thus be utilised to determine the dynamic balance between $\dot{Q}O_2$ and $\dot{V}O_2$ following the onset of exercise. In particular, the deoxyhaemoglobin (HHb) concentration ([HHb]) NIRS signal can be used to non-invasively estimate O₂ extraction in the skeletal muscle microcirculation. Thus, the NIRS-derived [HHb] signal would be expected to demonstrate a slower kinetic response at the onset of exercise if, as anticipated, eccentric exercise does compromise blood-muscle O₂ flux.

One recent investigation by Schneider et al. (2007) reported no change in $\dot{V}O_2$ kinetics at the onset of heavy intensity exercise 48 and 72 h following bench-stepping exercise designed to incur damage. An explanation for this apparent paradox, i.e. normal $\dot{V}O_2$ kinetics in the face of severe muscle damage and impaired microvascular haemodynamics, may be found in the work of Laaksonen et al. (2006) who reported that muscle damage increased muscle blood flow during exercise. If microvascular function and therefore the ability to match $\dot{Q}O_2:\dot{V}O_2$ effectively is compromised following eccentrically exercised muscle, it is possible that an elevation in the $\dot{Q}O_2:\dot{V}O_2$ ratio would serve to raise capillary O_2 pressures and restore blood-tissue O_2 flux in the face of capillary haemodynamic derangements. Accordingly, the present investigation utilised NIRS to test the novel hypothesis that eccentric exercise-induced muscle damage would elevate the muscle $\dot{Q}O_2:\dot{V}O_2$ ratio (as indicated by alterations in the [HHb] kinetics) during severe intensity exercise and thus preserve the speed of the $\dot{V}O_2$ kinetics at exercise onset.

7.3 Methods

Participants

Nine healthy men (mean \pm S.D. age 22.7 ± 2.8 years; height 1.83 ± 0.06 m; mass 76.7 ± 7.0 kg), asymptomatic of illness and pre-existing injury, volunteered to participate in the study. All were physically active but were not highly trained and had not undertaken any resistance training of the lower limbs for at least six months prior to assessment. Participants provided written informed consent to participate in the research which was approved by the Ethics Committee of the School of Sport and Health Sciences at the University of Exeter and conformed to the Declaration of Helsinki (See appendices B, C

and D for exemplar participant information sheet, participant consent form and ethical approval certificate). The participants were requested not to take any anti-inflammatory drugs for the duration of the study and to refrain from heavy exercise for 24 h prior to each visit.

Procedures

All testing was performed on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). The participants were instructed to report to the laboratory at the same time of day (± 1 h) on five separate occasions within a period of 2-3 weeks (Figure 7.1). On the first visit to the laboratory seat height and handlebar positions were individually adjusted for comfort and the adjustments were recorded and replicated in subsequent tests. The height and mass (SECA, UK) of each participant was also recorded.

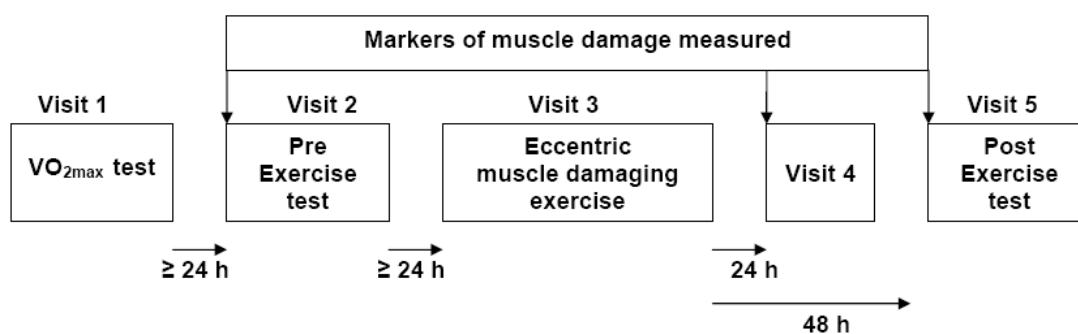


Figure 7.1 Schematic overview of experimental procedures. $\dot{V}O_{2\max}$, Maximal oxygen uptake.

Participants then completed an incremental exercise test to volitional exhaustion to determine maximal oxygen uptake ($\dot{V}O_{2\max}$) and gas exchange threshold (GET) and to establish future work intensities. This entailed cycling at a self-selected pedal rate (between 70 and 90 rpm) for 4 minutes of baseline cycling at 0 W. The work rate was then increased in a ramp fashion by 1 W every 2 s (i.e. $30 \text{ W}\cdot\text{min}^{-1}$) until the subject was unable to continue. The $\dot{V}O_{2\max}$ was defined as the highest 30-s mean value recorded before the subject's volitional termination of the test. The GET was determined independently by two experienced reviewers using the V-slope method (Beaver et al., 1986). The work rate that required 70% of the difference (Δ) between the GET and $\dot{V}O_2$ peak (severe exercise) was calculated, with account taken of the mean response time of the $\dot{V}O_2$ adaptation to ramp exercise (approximately 2/3 of the ramp rate i.e. minus 20 W) (Whipp, 1984). On the second and fifth visits, pre and 48 h after the eccentric muscle damaging exercise respectively, participants cycled at a self-selected pedal rate (between 70 and 90 rpm) for 4 minutes at 0 W, after which a warm-up phase at moderate intensity ($\sim 80\%$ GET) was applied for a further 6 minutes. After 2 minutes of passive rest, the subjects pedalled for 4 minutes of baseline cycling at 0 W after which the severe work rate was abruptly applied for 6 minutes. This test was repeated after a rest period of 2 hours. In the second test, the severe exercise bout was continued until the subject volitionally terminated the test at exhaustion.

Eccentric exercise

In order to provoke muscle damage, participants performed 100 (Smith) squats as 10 sets of 10 repetitions. The load on the bar was calculated to correspond to 70% of each participant's body mass. For further details of these procedures please refer to Chapter 3.

Measurements

Markers of muscle damage

Markers of muscle damage (muscle soreness and isokinetic peak torque) were measured immediately before and then 30 minutes and 48 hours after eccentric exercise. In addition plasma creatine kinase (CK) activity was assessed immediately before and then 30 minutes, 24 and 48 hours after eccentric exercise. Due to availability at 24 h, only 8 participants were measured for CK activity.

Assessment of muscle damage

All indicators of muscle damage, perceived muscle soreness (using a 0-10 visual analogue scale (VAS)), creatine kinase (CK) activity and isokinetic peak torque ($30 \text{ deg}\cdot\text{s}^{-1}$), were measured in the order listed, immediately before, and 24 and 48 h after performing the eccentric, muscle-damaging exercise protocol. For further details of these procedures please refer to Chapter 3.

Exercise test measures

Pulmonary gas exchange and ventilation were measured breath-by-breath throughout all tests with participants wearing a nose clip and breathing through a low-dead-space, low-resistance mouthpiece via an online gas analysis system (Cortex MetaMax 3B, Biophysik, Leipzig, Germany). The system was calibrated prior to every test in accordance with manufacturer's guidelines against known concentrations of cylinder gases (5% oxygen, 15% carbon dioxide) and a 3 l calibration syringe (Hans Rudolph, Kansas City, MO). The

$\dot{V}O_2$ data gathered from the pre- and post- exercise tests were subsequently modelled to provide estimates of the $\dot{V}O_2$ kinetic parameters (see *Modelling of $\dot{V}O_2$ and [HHb] data*).

Heart rate (HR), blood lactate concentration ([La]) and Ratings of Perceived Exertion (RPE) (Borg, 1998) were recorded at 2 min, 4 min and at the end of exercise. HR and [La] measures were also recorded during baseline cycling. HR was monitored using a wireless chest strap telemetry system (Polar Electro T31, Kempele, Finland) and measured continuously via a link to the Cortex gas analysis system. Fingertip blood samples were collected and analysed for [La] using an YSI 2300 STAT plus analyzer (Yellow Springs, Ohio, USA). Participants were familiarized with Borg's 6-20 RPE Scale and provided with standardised instructions on how to employ the scale (Borg, 1998). Participants were encouraged to focus on their overall perception of exertion when reporting their RPE.

Near-infrared spectroscopy (NIRS)

Oxygenation profiles of the right *vastus lateralis* muscle were recorded in all exercise tests using a continuous wave near-infrared spectrometer (NIRS) (Hamamatsu NIRO 300, Hamamatsu Photonics KK, Japan) (Figure 7.2). The system monitored concentration changes in oxyhaemoglobin (HbO₂) and deoxyhaemoglobin (HHb) which were calculated from the light attenuation change by utilising the modified Beer-Lambert law. The HHb concentration ([HHb]) signal obtained from the NIRS was regarded as being relatively insensitive to blood volume changes during exercise and thus reflected the balance between the delivery and utilisation of oxygen (Ferrari et al., 1997).

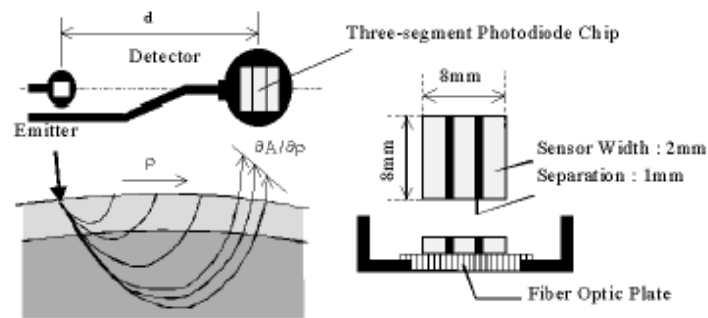


Figure 7.2 Measurement principle and probe structure of the NIRO 300 (Hamamatsu, Hamamatsu Photonics KK, Japan)

Pulsed light was emitted at 1 s intervals from the emission probe at four different wavelengths (775, 810, 850 and 910nm) and was detected, as a function of distance, using a three segment photodiode detection probe that received NIRS signals at 2 Hz. The probes were housed in the black silicone holder provided. The inter-optode spacing between emitter and receiver was 4 cm, and the penetration depth was approximately half of the distance between the emitter and the receiver, i.e. 2 cm. Prior to placement on the right *vastus lateralis*, the site was shaved and cleaned using an alcohol swab. The NIRO300 system was then calibrated and the probe holder secured by means of a double-sided adhesive sheet ~12 cm above the lateral epicondyle of the right leg, with the location marked using an indelible marker pen to enable reproduction of the probe positions in subsequent tests (48 h). The thigh with attached probe holder was then wrapped in a dark-coloured, elastic bandage to further secure the probes and to eliminate ambient light that might contaminate the NIRS signal (Figure 7.3)

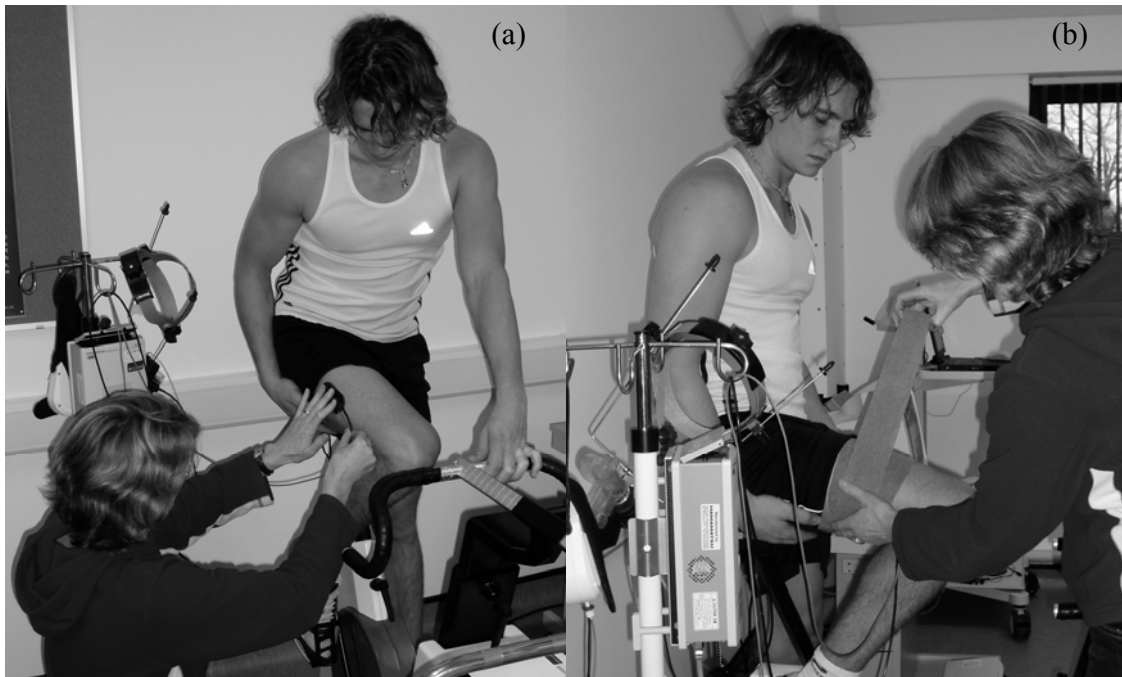


Figure 7.3. Attachment of the NIRS probes. The NIRS probe holder is secured by means of a double-sided adhesive (a). The thigh with attached NIRS probe holder is wrapped in an elastic bandage (b).

The NIRS data gathered represented relative concentration changes in the haemoglobin chromophores and were, therefore, not representative of absolute tissue O_2 values. As [HHb] was measured as a change from baseline values the probe gain was zero set prior to testing with the subject at rest in a seated position. Differences in the thickness of the overlying adipose tissue may influence the amplitude of the NIRS signal. However, the same subjects were employed pre- and post eccentric exercise and the probe positions were rigorously maintained for each subject in each test, thus no correction for inter-site adiposity was necessary. Following exercise testing the data were downloaded and the resulting text files stored for subsequent analysis.

Modelling of $\dot{V}O_2$ and [HHb] data

The breath-by-breath data from each exercise test were filtered manually to remove outlying breaths, defined as breaths ± 3 SD from the adjacent five breaths. The data for each individual were then interpolated to provide 1 s values and the two data sets from each of the pre- and post- exercise tests were time-aligned and averaged. The first 20 s of data after the onset of exercise (the Phase I response) were deleted and a biexponential model was used to analyze the $\dot{V}O_2$ responses to severe exercise, as described by the following equation:

$$\begin{aligned}\dot{V}O_2(t) = & \dot{V}O_{2\text{baseline}} \\ & + Ap[1 - e^{-(t - T_{dp})/\tau_p}] \quad (\text{phase 2 / primary component}) \\ & + As[1 - e^{-(t - T_{ds})/\tau_s}] \quad (\text{phase 3 / slow component})\end{aligned}$$

where t is time; $\dot{V}O_{2\text{baseline}}$ is baseline $\dot{V}O_2$; Ap and As are the primary and slow component amplitude, respectively; T_{dp} and T_{ds} are the primary and slow component time delays, respectively; and τ_p and τ_s are the time constants of the primary and slow components respectively. The parameters of the model were determined by using a nonlinear least squares algorithm. In the equations above, $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t , and $\dot{V}O_{2\text{baseline}}$ represents the average $\dot{V}O_2$ through the baseline cycling period. Because the time to exhaustion was not identical in the first and second bouts of severe exercise, we fitted the data 1) to the end of exercise in both bouts and 2) to the same point in time (given by the time to exhaustion in the shortest bout). The primary

component “gain” (i.e., $A_p/\Delta WR$) was calculated from the projected asymptotic $\dot{V}O_2$. In addition, the “actual” gain attained at the end of exercise was calculated.

To provide information on the effect of eccentric muscle-damaging exercise on the dynamics of muscle oxygenation, we also modelled the $\Delta[HHb]$ response to severe exercise. The NIRS-derived $[HHb]$ data were time-aligned and averaged to provide a single response for each subject pre- and post- eccentric exercise. The time delay before an increase in $[HHb]$ after exercise onset was determined as the first point greater than one standard deviation above the mean of the baseline (DeLorey et al., 2003). $[HHb]$ data were then fitted with a bi-exponential model similar to that described by the equation above, with the exception that the fitting window started at the onset of exercise (i.e., at *time 0*). Subsequently, $[HHb]$ data were fitted with a mono-exponential model from the onset of exercise to the time point representing the interface of the primary and slow component to determine the rate of adaptation of muscle deoxygenation during the primary phase (MRT_1). In addition, the $[HHb]$ dynamics for the entire response were modelled with a similar mono-exponential function (MRT_1).

Statistical analysis

Changes in the markers of muscle damage (peak torque, soreness and CK activity) were analysed using a series of one-way repeated measures (RM) ANOVA. All data were checked for assumptions of normality. As the CK activity data were found not to be normally distributed, the values were log-transformed prior to statistical analysis (Twist & Eston, 2005) (see appendix H).

Following transformation CK activity data were normally distributed. Changes in HR, RPE, [La] and ventilation were analysed using separate 2-way RM ANOVAs (test x time). Assumptions of sphericity were evaluated using Mauchly's test. Where sphericity was violated ($P < 0.05$) the Greenhouse-Geisser (GG) correction factor was applied. Post-hoc Tukey tests modified for repeated measures (Stevens, 2002) were run to determine where significant differences occurred. Paired t-tests were used to determine significant differences in time-to-exhaustion and the $\dot{V}O_2$ and [HHb] kinetic responses to severe intensity exercise before and after eccentric exercise. All data were analysed using the statistical software package SPSS for Windows (version 13). Statistical significance was set at 0.05.

7.4 Results

Markers of muscle damage

The eccentric exercise was effective in provoking significant changes in all markers of muscle damage. Table 7.1 shows changes in isokinetic peak torque, perceived muscle soreness and plasma CK activity before and at 24 h and 48 h post eccentric exercise.

Table 7.1 Changes in markers of muscle damage. Mean \pm SD values before (pre) and at 24 h and 48 h after eccentric exercise.

Measured variable	pre	24 h post	48 h post
Peak Torque (N·m) 30 deg.s⁻¹	287 \pm 39	227 \pm 44*	228 \pm 60*
Soreness	0.6 \pm 0.5	6.4 \pm 1.8*	7.1 \pm 1.5*
CK activity (U/L)	172 \pm 123	740 \pm 666*	373 \pm 208

Values are means \pm SD before (pre) and 24 h and 48 h after eccentric exercise (post). Soreness, visual analogue scale 0-10. CK, creatine kinase. *significantly different ($P < 0.05$) from pre value

Isokinetic peak torque (30 deg.s⁻¹) decreased by 21% at 24 h post-eccentric exercise and remained depressed at 48 h ($F_{(2,16)} = 21.85$ $p < 0.001$). Significant soreness was reported 24 h after eccentric exercise with the highest values at 48 h ($F_{(2,14)} = 80.50$ $p < 0.001$). Plasma CK activity increased after eccentric exercise, with the highest activity observed at 24 h ($F_{(2,16)} = 17.15$ $p < 0.001$). Changes in markers of muscle damage were detected in all subjects, although considerable inter-subject variability was observed. Peak decrements in isokinetic peak torque (30 deg.s⁻¹) ranged from 12 - 44%, and peak increases in soreness and plasma CK activity ranged from 53 - 95% and 146 - 1176%, respectively.

Response to severe intensity exercise

Table 7.2 shows the $\dot{V}O_2$ responses to severe intensity exercise. There were no changes in the phase II $\dot{V}O_2$ kinetics following eccentric exercise, nor was there a change in the slow component time delay (TD) ($P > 0.05$). However the amplitude of the slow component was significantly reduced ($t_{(8)} = 3.84$, $P < 0.05$) and the overall mean response time (MRT) was significantly faster ($t_{(8)} = 4.01$, $P < 0.05$) following eccentric exercise. A significantly shorter time to exhaustion was observed in the 2nd bout of severe intensity exercise (pre:

7:24 min:s (\pm 2:41) post 6:14 min:s (\pm 2:46)) ($t_{(8)} = 2.58$, $P < 0.05$). The $\dot{V}O_2$ response of a representative subject is illustrated in Figure 7.4.

Table 7.2 Pulmonary O_2 uptake responses to severe intensity exercise before and after eccentric muscle damaging exercise.

	Pre	48 h post
Primary component		
TD _p (s)	12 \pm 4	12 \pm 2
τ_p (s)	25 \pm 4	24 \pm 3
Ap $\dot{V}O_2$ (L \cdot min ⁻¹)	2.36 \pm 0.23	2.37 \pm 0.23
Gain (mL \cdot min ⁻¹ \cdot W ⁻¹)	9.02 \pm 0.45	9.04 \pm 0.52
Slow component		
TD ₂ (s)	102 \pm 19	107 \pm 29
A _{2ref} $\dot{V}O_2$ (L \cdot min ⁻¹)	0.63 \pm 0.33	0.37 \pm 0.17*
A _{2end} $\dot{V}O_2$ (L \cdot min ⁻¹)	0.67 \pm 0.33	0.38 \pm 0.17*
Overall response		
MRT _{ref}	61 \pm 15	51 \pm 9*
MRT _{end}	64 \pm 15	51 \pm 9*
Peak $\dot{V}O_2$ (L \cdot min ⁻¹)	3.89 \pm 0.44	3.70 \pm 0.36

Values are means \pm SD. TD_p, τ_p , Ap and gain are the time delay, time constant, amplitude and increase in $\dot{V}O_2$ per unit increase in work rate for phase II kinetics respectively. TD₂, A_{2ref} and A_{2end} are the time delay, amplitude to 6 mins and amplitude to end of exercise for the slow component, respectively. MRT_{end} and MRT_{ref} are the mean response times fitted to the end of exercise in both bouts and to the same point in time (given by the time to exhaustion in the shortest bout), respectively. Peak $\dot{V}O_2$ tended to be lower post-eccentric exercise but the difference was not statistically significant. Significant difference ($P < 0.05$) from pre value.

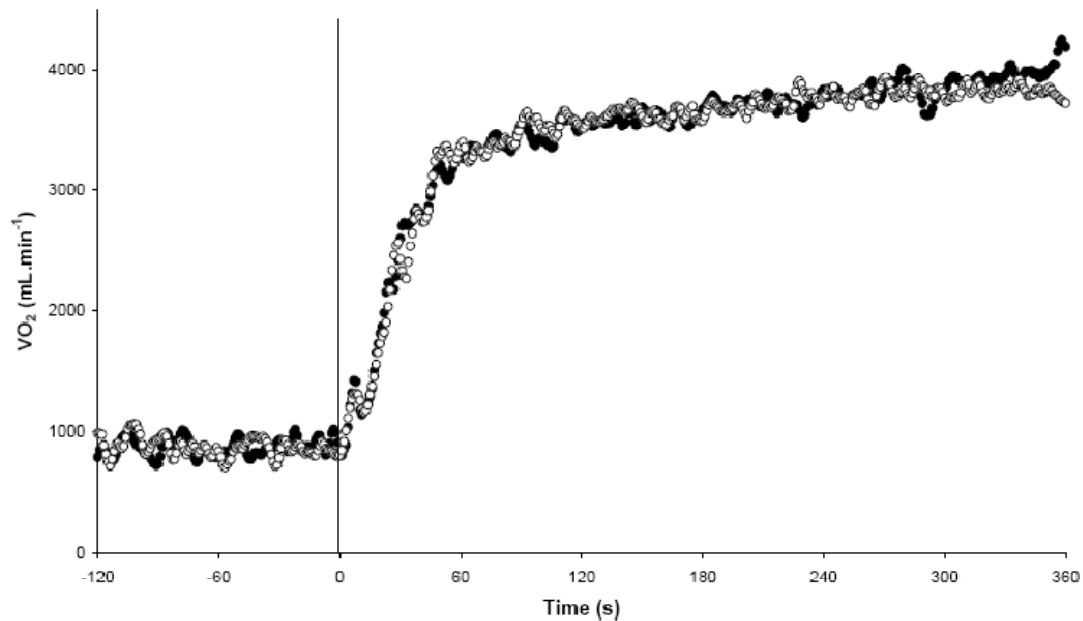


Figure 7.4 A representative subject's $\dot{V}O_2$ response to severe cycle exercise pre- (●) and 48 h post- (○) eccentric exercise. The vertical line represents the transition from 'unloaded' to 'loaded' cycling.

There was a significant increase in RPE values reported during severe intensity exercise following eccentric exercise ($F_{(1,8)} = 6.7$ $P < 0.05$). However there were no significant differences in the blood lactate or heart rate responses before and after eccentric exercise (Table 7.3) ($P > 0.05$). In addition, there was a significant increase in the ventilatory equivalent for O_2 ($\dot{V}O_2$) following eccentric exercise (pre: $29.3 (\pm 3.5)$ post: $32.6 (\pm 5.2)$) ($F_{(1,8)} = 7.45$, $P < 0.05$).

Table 7.3 Ratings of Perceived Exertion (RPE), Heart Rate (HR) and blood lactate concentration ([La]) during severe intensity exercise pre- and 48 h post-eccentric exercise.

	<u>baseline</u>		<u>min 2</u>		<u>min 4</u>		<u>end exercise</u>	
	pre	48 h	pre	48 h	pre	48 h	pre	48 h
RPE*	NA	NA	15 ± 1	16 ± 1	17 ± 1	18 ± 2	19 ± 1	19 ± 1
HR (b·min⁻¹)	83 ± 9	86 ± 6	159 ± 8	163 ± 11	174 ± 11	176 ± 11	184 ± 9	181 ± 11
[La] (mmol·l⁻¹)	0.9 ± 0.5	1.0 ± 0.4	3.5 ± 1.0	3.8 ± 1.0	6.9 ± 1.3	6.8 ± 1.8	8.4 ± 1.4	8.7 ± 1.8

Values are means ± SD. RPE, ratings of perceived exertion based on Borg's 6-20 scale (Borg, 1998); HR, heart rate; [La], blood lactate concentration; NA, not applicable.

* Significant main effect for time ($P < 0.05$).

There was also a significant interaction of time x test ($F_{(9,72)} = 3.15$, $P < 0.05$) on $\dot{V}_E/\dot{V}O_2$.

Post-hoc Tukey tests indicated that $\dot{V}_E/\dot{V}O_2$ was significantly greater post eccentric exercise for the last 70% of exercise with mean values rising from 25.3 to 36.0 (pre) and from 26.7 to 39.9 (post) ($P < 0.05$).

The results of the kinetic response of [HHb] to severe intensity exercise pre- and 48 h post-eccentric exercise are shown in Table 7.4. Most importantly, with respect to our experimental hypothesis, both the [HHb] MRT_1 and MRT_i were significantly slower following eccentric exercise ($P < 0.05$). There was no significant correlation between any of the markers of muscle damage and indices of muscle oxygenation in the post damage condition ($P > 0.05$). The [HHb] response of a representative subject is illustrated in Figure 7.5.

Table 7.4 [HHb] response to severe intensity constant-load exercise pre- and 48 h post-eccentric exercise.

	Pre	48 h
MRT₁ (s)	14 ± 3	19 ± 3*
Primary Amp (%)	91 ± 8	88 ± 8
Primary Amp (A.U.)	309 ± 102	297 ± 72
MRT_t (s)	16 ± 4	21 ± 4*
SC Amp (%)	9 ± 8	12 ± 8
SC Amp (A.U.)	33 ± 25	41 ± 25

Values are means ± SD. [HHb], deoxyhaemoglobin concentration; MRT₁ and MRT_t are the mean response time of the primary phase (the rate of adaptation of muscle deoxygenation during the primary phase) and the overall response (the rate of adaptation of muscle deoxygenation for the entire response), respectively. Primary Amp is the change in [HHb] during the initial, fast increase expressed as a percentage of the overall response (%) and as arbitrary units (A.U.). SC Amp is the change in [HHb] during the slow component (the additional increase in [HHb] which develops slowly when exercise intensity is above the gas exchange threshold) expressed as a percentage of the overall response (%) and as arbitrary units (A.U.)
 *48 h post value significantly slower than pre value ($P < 0.05$).

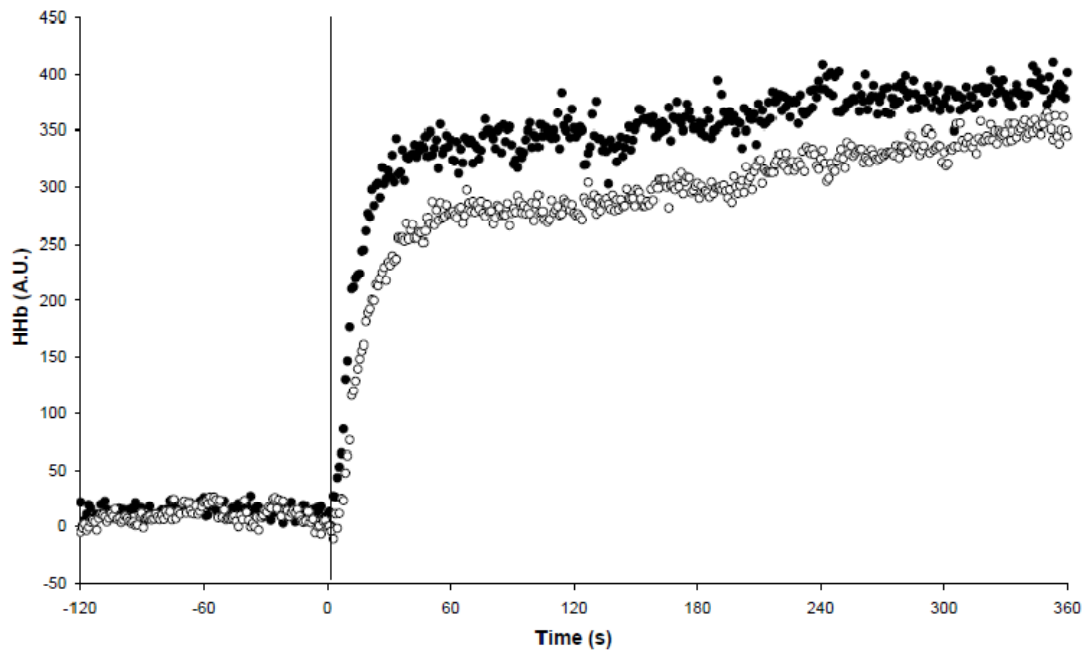


Figure 7.5 Deoxygenated Hb ([HHb]) response to severe cycle exercise pre- (●) and 48 h post- (○) eccentric exercise in a representative subject. The vertical line represents the transition from unloaded to loaded cycling. Note the slower overall [HHb] kinetics following eccentric exercise (the slower rate of adaptation of muscle deoxygenation or fractional O₂ extraction, for the entire response),. AU, arbitrary units.

The altered $\dot{Q}O_2:\dot{V}O_2$ balance is most pronounced during the initial response following the onset of severe intensity exercise (Figure 7.6), with the greatest mean difference observed over the first 5-20 s (Figure 7.7). There were no significant differences in the pre- and post-conditions between the amplitude of the response in either the primary phase or slow component ($P > 0.05$). Similarly there was no difference between the total haemoglobin responses in the two conditions (Figure 7.8).

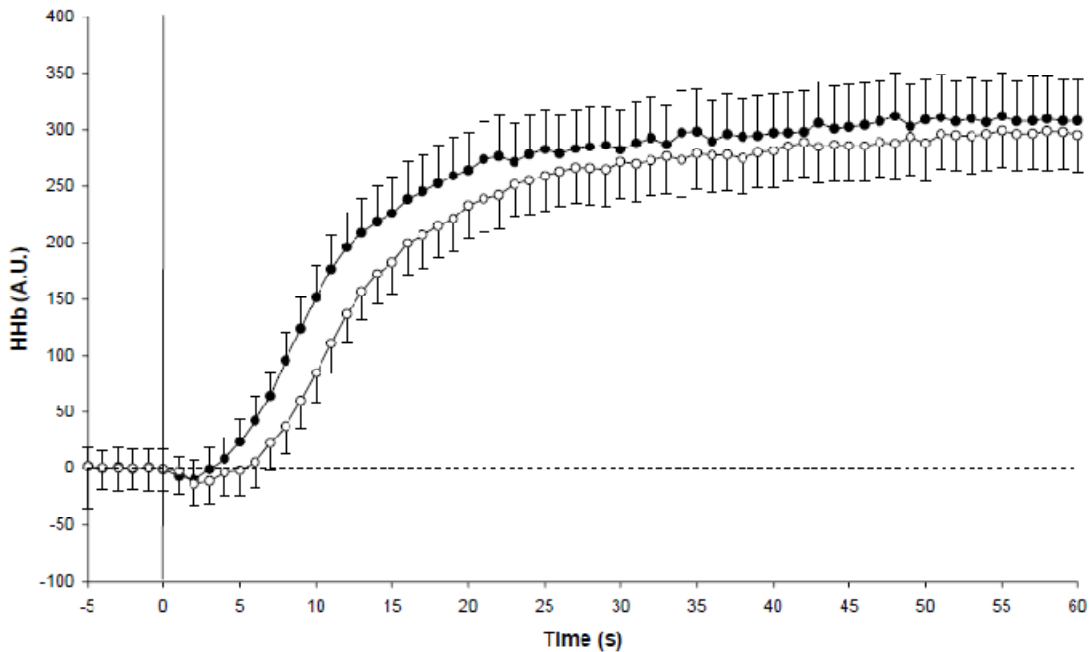


Figure 7.6 Mean initial [HHb] response to severe cycle exercise pre- (●) and 48 h post- (○) eccentric exercise. The vertical line represents the transition from ‘unloaded’ to ‘loaded’ cycling. The data are normalized for amplitude of the response at baseline. Values are mean (\pm SE). AU, arbitrary units.

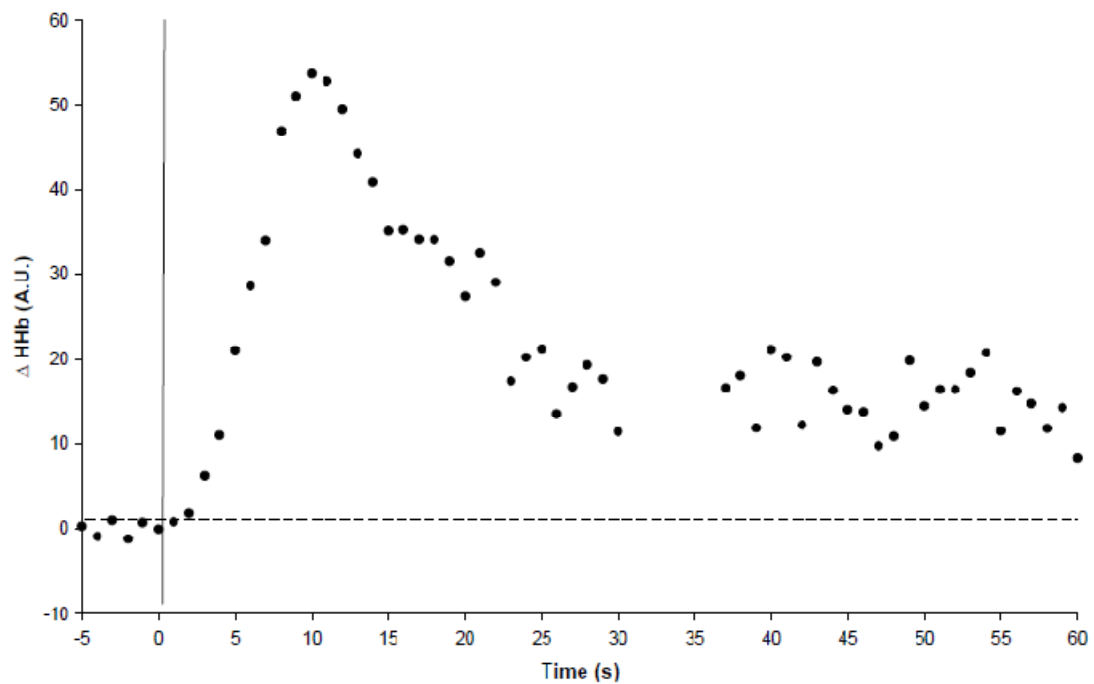


Figure 7.7 Mean difference in the initial [HHb] response to severe intensity exercise pre- and 48 h post eccentric exercise (i.e. pre- minus post-eccentric exercise data). AU, arbitrary units.

7.5 Discussion

The principal original finding of this investigation is that eccentric, muscle-damaging exercise results in a slowing of muscle [HHb] kinetics without altering pulmonary $\dot{V}O_2$ kinetics during high-intensity cycle exercise in humans. We interpret the slower [HHb] kinetics, in the face of unchanged pulmonary $\dot{V}O_2$, to be consequent to a local elevation of the $\dot{Q}O_2:\dot{V}O_2$ ratio. The observation that the [HHb] kinetics were over 30% slower 48 h after the performance of eccentric exercise, suggests that the matching of $\dot{Q}O_2$ and $\dot{V}O_2$ was profoundly altered as a consequence of the intervention.

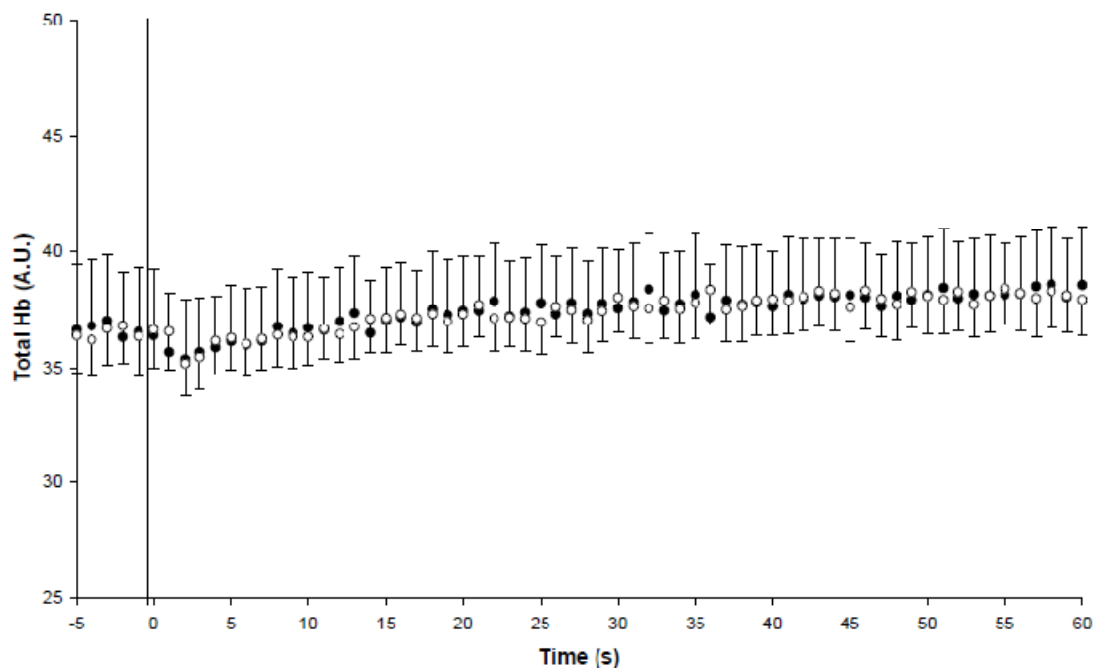


Figure 7.8 Total haemoglobin response to severe cycle exercise pre- (●) and 48 h post- (○) eccentric exercise. AU, arbitrary units. Changes in total haemoglobin were not different between conditions such that changes in [HHb] provided valid measures of muscle deoxygenation (fractional O₂ extraction). Increased blood flow, such as has been observed by Laaksonen et al. (2006) could compensate for the decreased proportion of capillaries supporting RBC flow such as has been observed by Kano et al. (2005) and therefore maintain the total haemoglobin response in the area of interrogation.

The dynamic balance between O₂ delivery and O₂ utilisation has been keenly debated with regard to possible limitations to muscle $\dot{V}O_2$ kinetics (Poole et al., 2008). During transitions to exercise intensities below GET, the compelling weight of evidence supports the premise that metabolic inertia is the principal limitation to muscle O₂ uptake (Bangsbo et al., 2000; Grassi et al., 1998, 1996). However, for transitions to exercise intensities above GET, the delivery of O₂ may exert an additional modest constraint on muscle $\dot{V}O_2$ kinetics (Grassi et al., 2000, 2003; Tschakovsky & Hughson, 1999). The present data indicate that following eccentric exercise-induced muscle damage, $\dot{V}O_2$ kinetics are preserved by an

increase in local muscle blood flow which is presumably necessary to compensate for microcirculatory dysfunction (Kano et al., 2005). Increased blood flow, such as has been observed by Laaksonen et al. (2006) following eccentric exercise, could compensate for the decreased proportion of capillaries supporting RBC flow such as has been observed by Kano et al. (2005). In support of this thesis, we observed no change in the total haemoglobin response, indicative of local muscle blood flow in the area of interrogation. Thus, the changes in the [HHb] response provided valid measures of muscle deoxygenation (fractional O₂ extraction). These data therefore imply that the subjects were operating to the right of the 'tipping point' (Poole et al., 2008) during the exercise bout, such that compensatory changes in local muscle blood flow were able to prevent a measurable slowing of $\dot{V}O_2$ kinetics following muscle damage.

The squatting protocol employed herein (Byrne & Eston, 2002a, 2002b), was effective in inducing muscle damage as indicated by the significant decreases in peak torque and increases in plasma CK activity. Despite this, there was no significant change in phase II $\dot{V}O_2$ kinetics following eccentric exercise. These data are consistent with Schneider et al. (2007) who reported that the $\dot{V}O_2$ and HR responses to heavy-intensity cycling exercise were unaffected by prior eccentric exercise and surmised that a moderate degree of muscle damage did not impair O₂ delivery to the active muscle or alter the $\dot{Q}O_2/\dot{V}O_2$ ratio. However, indices of muscle oxygenation were not measured by these authors (60). Our findings contrast with those of Ahmadi et al. (2008), who reported faster oxygen desaturation (analogous to a *lower* $\dot{Q}O_2/\dot{V}O_2$ ratio) during isometric contractions at 30%, 50% and 80% MVC following exhaustive downhill walking. However, differences in the

experimental protocol and exercise modalities employed make it very difficult to compare the two studies. The high intramuscular pressure generated during isometric contractions is known to impede muscle blood flow and this, or an increase in the energy demand of contraction, might account for the faster oxygen saturation reported by Ahmadi et al. (2008).

The experimental protocol used in the present study differs fundamentally from that employed by Kano et al. (2005). These authors electrically induced twitch muscle contractions (1 Hz, 3–5 V, 2-ms pulse duration) in rat spinotrapezius muscle, whereas in our study, human subjects performed dynamic high-intensity cycle-exercise transitions. Whilst taking these differences into consideration, it is important to note that rat spinotrapezius muscle does provide a highly acceptable, comparative model for the analysis of human microvascular and myocyte damage as it exhibits a fibre composition (Delp & Duan, 1996) and oxidative capacity (Leek et al., 2001) that closely resemble that of the human quadriceps. Thus the disruption observed in rat microvasculature subsequent to eccentric exercise would, most likely, also be present in the damaged muscle of subjects interrogated herein. Kano et al. (2005) reported disrupted capillary geometry and substantial microvascular dysfunction following eccentric exercise. Specifically, an increase in the capillary luminal area of damaged muscle was reported which resulted in a decreased PO_2 mv at the onset of electrically stimulated contractions. In addition a 27–34% increase in non-RBC-flowing capillaries was found 1–3 days after a single bout of eccentric exercise.

Such structural and functional alterations to the microvasculature might act to alter the matching of $\dot{Q}O_2$ to $\dot{V}O_2$ both spatially and temporally with respect to tissue energetic requirements. Specifically, the lower [HHb] observed at any given $\dot{V}O_2$ across the transition post-eccentric exercise suggests that achievement of the requisite blood-myocyte O_2 flux might demand a higher microvascular O_2 driving pressure. Blood-muscle O_2 flux is primarily determined by the number of erythrocytes lying adjacent to active myocytes at any given time (Federspiel & Popel, 1986; Groebe & Thews, 1990). A decrease in the proportion of capillaries supporting RBC flow would lead to a reduction in blood-muscle O_2 flux. Similarly, an increase in the diameter of free-flowing capillaries would increase the carrier-free diffusion distance and lead to a reduction in O_2 diffusing capacity.

Fick's law of diffusion states that:

$$\dot{V}O_{2m} = DO_2 (PO_{2mv} - PO_{2intra\text{myocyte}})$$

where DO_2 is the diffusing capacity for O_2 and PO_{2mv} is the microvascular O_2 pressure. Such reductions to DO_2 would be expected to negatively impact $\dot{V}O_2$ particularly in the presence of concomitant alterations to PO_{2mv} similar to those reported by Kano et al. (2005). The O_2 pressure gradient from blood to myocyte which drives O_2 diffusion is determined principally by alterations of PO_{2mv} (Poole et al., 2006; Poole & Ferreira, 2007). Thus the accelerated fall of PO_{2mv} observed by Kano and colleagues during the first 20–40 s of electrically stimulated muscle contractions would be expected to have a profound influence on the muscle O_2 diffusing capacity and result in a slowing of $\dot{V}O_2$

kinetics (Behnke et al., 2004, 2007, 2006). However, the PO_{2mv} herein (as judged by the [HHb] response) appeared to be elevated during the dynamic rest-exercise transitions post-eccentric exercise and the $\dot{V}O_2$ kinetic response remained unchanged.

A given $\dot{V}O_2$ is achieved through the interaction of O_2 delivery ($\dot{Q}O_2$) and O_2 diffusing properties (Behnke et al., 2003). Additionally, changes in pulmonary $\dot{V}O_2$ across the rest-exercise transition are known to directly reflect leg $\dot{V}O_2$ during cycling exercise (Poole et al., 1991), and demonstrate a good approximation of the muscle $\dot{V}O_2$ kinetics (Grassi et al., 1996). Therefore, the elevated $\dot{Q}O_2:\dot{V}O_2$ ratio evidenced by the slower [HHb] kinetic response (i.e. lower [HHb] at a given $\dot{V}O_2$ across the exercise transition) must, in this instance, be due to compensatory changes to O_2 delivery (see Appendix J). Pertinent to this issue, increased blood flow (and therefore increased O_2 delivery) has been observed by Laaksonen et al. (2006) who found that blood flow to the exercising *Quadriceps femoris* was elevated by 25% after a prior bout of exhaustive eccentric exercise. Importantly, they also reported that $\dot{V}O_2$ remained unchanged and suggested that the altered $\dot{Q}O_2:\dot{V}O_2$ balance observed may have been due to impaired oxygen extraction. Thus, following eccentric muscle-damaging exercise, adaptive, compensatory mechanisms may act to elevate PO_{2mv} across the rest-exercise transition, preserving blood-myocyte O_2 flux as indicated by the unchanged phase II $\dot{V}O_2$ kinetics.

The higher ratings of perceived exertion reported for a given exercise intensity (70 % Δ) following eccentric exercise may be due, in part, to the enhanced ventilatory response which accompanies the increased muscle soreness. It has been proposed that an altered

sense of effort may be part of a central protective mechanism whereby neural inhibition serves to reduce force generation in order to prevent further injury (Kyrolainen et al., 2000; Michaut et al., 2002; Proske et al., 2004). Additionally, Hotta et al. (2006) suggested that changes in neural factors contribute not only to alterations in force generation but also to an enhanced ventilatory response. Group III and IV afferent fibres located in and around the blood vessels of exercising muscle are involved in modulating the ventilatory response. Distension of these blood vessels provokes a discharge from the afferent fibres that leads to an increase in ventilation (Haouzi et al., 2004, 1999). Thus, neural monitoring of peripheral vascular events might account, in part, for the enhanced $\dot{V}O_2$ observed in this study if there were alterations to the microvasculature as a result of eccentric exercise. Pertinent to this issue, in studies employing male Wistar rats, Kano et al. (2004) have reported changes in the capillary lumen shape (luminal ellipticity) that increase the luminal cross-sectional area by up to 62% following eccentric exercise. Similar increases in the cross-sectional area of the microvessels in the muscles recruited in the present investigation might serve to augment the ventilatory response via neural modulation.

It has been proposed that, following eccentric exercise, alterations to motor unit recruitment patterns arise in order to meet the energetic demands of a given work rate (Clarkson, 1992). Reports of elevated blood lactate concentration following eccentric exercise have been attributed to the additional recruitment of type II fibres and a concomitant rise in the rate of glycogenolysis (Chen et al., 2007b; Gleeson et al., 1998). However, Chen (2003) reported that neural adaptation to motor unit activation patterns occurred after a single bout of eccentric exercise, such that additional type I motor units were recruited. Additional fibre recruitment has been implicated in the development of the $\dot{V}O_2$ slow component in high-

intensity exercise (Krustrup et al., 2004). Specifically, it has been proposed that the development of a $\dot{V}O_2$ slow component may be related, in part, to the recruitment of additional type II fibres (Barstow et al., 1996; Pringle et al., 2003; Whipp, 1994). The reduction in the amplitude of the $\dot{V}O_2$ slow component observed herein may therefore be indicative of an altered motor unit recruitment pattern consequent to muscle damage (Pringle et al., 2003). However, the unchanged primary $\dot{V}O_2$ response and the unchanged blood lactate concentration observed are arguably not in keeping with this suggestion. Additionally, full expression of the slow component may have been impeded as a result of muscular fatigue and a reduction in maximal work capacity brought about by the effects of eccentric exercise. Such impediments to performance have previously been reported to result in a shorter time to exhaustion in mice (Carmichael et al., 2005, 2006) and a reduced time trial running distance in human subjects (Marcora & Bosio, 2007). Accordingly, the reduction in the $\dot{V}O_2$ slow component observed post-eccentric exercise may be attributed to a shorter time to exhaustion and a tendency for a lower $\dot{V}O_2$ peak to be achieved in this condition.

Experimental considerations

As mentioned earlier, our findings contrast with those of Kano et al. (2005) due to fundamental differences in the muscle activation processes employed rather than species variation. We studied dynamic exercise transitions to severe intensity (70 % Δ) cycle exercise, whereas Kano and colleagues utilised a set rate of electrical stimulation (1 Hz, 3–5 V, 2-ms pulse duration) to induce muscle contractions. Electrical stimulation induces recruitment of all fibres, whilst voluntary exercise recruits specific fibres and fibre types

dependant on exercise intensity and duration (Kindig et al., 2002). Thus, the muscle activation and attendant fibre recruitment patterns studied herein present a more ecologically valid model for investigation. As such, this study may provide a more realistic insight into the effects of eccentric muscle-damaging exercise on functional human performance.

NIRS is an established technique for the measurement of muscle oxygenation (e.g. Ferrari et al., 2004; Jones et al., 2006). However, the reliability and reproducibility of the NIRS-derived [HHb] signal is dependant on the precise placement of the optodes. In the present study, optode location was marked on each individual subject during the first visit to the laboratory and placement was carefully reproduced on subsequent visits. Koga et al. (2007) have revealed the presence of significant heterogeneity with respect to the dynamics of muscle oxygenation within the quadriceps muscles of healthy subjects following the onset of exercise. These findings are not surprising given that muscle blood flow, motor unit distribution and recruitment and consequently vascular responses are known to be heterogeneous within and across muscles. However, it is important to recognise that the NIRS data reported herein is representative of changes within the superficial muscle area under interrogation only and as such may not be representative of the entire muscle mass affected by eccentric exercise.

7.6 Conclusion

In conclusion, the present investigation suggests that eccentric, muscle-damaging exercise alters the matching of $\dot{Q}O_2$ and $\dot{V}O_2$ during severe intensity exercise. Specifically, across the rapid metabolic transition following the onset of exercise, for a given $\dot{V}O_2$, the [HHb] signal is reduced. We propose that structural and possibly functional alterations to the microvasculature act to increase the $\dot{Q}O_2:\dot{V}O_2$ ratio both spatially and temporally with respect to tissue energetic requirements. Accordingly, following eccentric muscle-damaging exercise, compensatory mechanisms act to elevate the microvascular driving pressure for blood-myocyte O_2 flux, enabling a preservation of the kinetics of $\dot{V}O_2$ across the rest-to-exercise transition.

CHAPTER 8
CONCLUSIONS

8.1 Main Findings

8.1.1 The ventilatory response to dynamic exercise with EIMD

Previous investigations have reported that the ventilatory response to running exercise is unchanged following EIMD (Paschalis et al., 2005; Scott et al., 2003; Marcora and Bosio, 2007). Other investigations that reported an increased ventilatory response and an elevated blood [La] response during running attributed the changes to altered lower limb kinematics (Braun & Dutto, 2003; Chen et al., 2007b, 2008). During cycle exercise, where the potentially confounding influence of altered kinematics is avoided, increases in both ventilation and blood [La] have been reported (Gleeson et al., 1995; Schneider et al., 2007). Prior to the studies which comprise this thesis being undertaken, Schneider et al. (2007), using an exercise intensity of 40% Δ , provided the only example of an investigation using a specific exercise domain to study the influence of EIMD on physiological responses to dynamic exercise.

The findings of studies 1 (Chapter 4) and 4 (Chapter 7) demonstrated that the ventilatory response to cycle exercise was elevated 48 h after eccentric exercise (100 squats with a load corresponding to 70% of body mass). Importantly, ventilation was increased not only during severe intensity (70% Δ) cycle exercise but also during moderate intensity (80% GET) cycle exercise without any significant change in the blood [La] response. Previously, it had been assumed that an increase in [La] contributed to an augmented ventilatory response. However, our findings, in particular the observation that the ventilatory response following EIMD is higher during exercise below GET, suggest that the altered exercise response may not be due to changes in metabolic factors. Rather, we propose that neural monitoring of peripheral vascular and local muscular events may, in part, account for the

augmented ventilatory response observed. The mechanisms involved in ventilatory control during exercise are complex and involve a combination of central command and afferent feedback. However, the structural and functional changes that characterise EIMD are understood to augment discharge from group III and IV afferents. These changes rather than elevated blood [La] which is not an obligatory consequence of EIMD, most probably contribute to the greater ventilatory response to dynamic cycle exercise evoked by EIMD.

8.1.2 The Gas Exchange Threshold (GET) and EIMD

During dynamic incremental exercise protocols, identification of the GET provides the basis for the non-invasive estimation of the lactate threshold (Tlac) (Beaver et al., 1986; Caiozzo et al., 1982; Wasserman et al. 1973, 1990). However, the ventilatory and lactate responses to incremental exercise may be dissociated by various experimental and clinical conditions (e.g. Poole & Gaesser, 1985; Hughes et al., 1982; Hagberg et al., 1982). It was our contention that EIMD was likely to exacerbate the ventilatory response to incremental/ramp exercise and, in so doing, potentially dissociate the ventilatory, gas exchange and lactate responses.

The findings of study 2 (Chapter 5) demonstrated that 48 h after eccentric exercise GET occurred at a lower work rate (pre, 136 ± 27 W; post, 105 ± 19 W, $P < 0.05$) and $\dot{V}O_2$ (pre, 1.58 ± 0.26 ; post, 1.41 ± 0.14 l.min⁻¹, $P < 0.05$). However, the lactate threshold occurred at a similar work rate (pre, 161 ± 19 W; post, 158 ± 22 W, $P > 0.05$) and $\dot{V}O_2$ (pre, 1.90 ± 0.20 l.min⁻¹; post, 1.88 ± 0.15 l.min⁻¹, $P > 0.05$) after eccentric exercise. These findings demonstrated that EIMD dissociates the ventilatory response to incremental exercise from the blood [La] response. Thus, these findings provide further evidence to support the thesis

that ventilation may be controlled by additional or altered neurogenic stimuli following eccentric exercise.

8.1.3 The perception of exertion during dynamic exercise with EIMD

Irrespective of the mode of damage, the exercise intensity, or exercise protocol employed, eccentric, muscle-damaging exercise is known to evoke an increased sense of effort (Gleeson et al., 1995; Scott et al., 2003; Marcora & Bosio, 2006; Twist & Eston, 2009). The cues which inform the perceptual response to exercise may arise from central or peripheral sensations, with the influence of central (cardiorespiratory) cues of lesser importance than that of local (muscle) sensations, particularly at lower exercise intensities (Mihevic, 1981; Hampson et al., 2001). As such, the perception of exertion reported during exercise with EIMD may be differentially influenced by central and peripheral cues dependent on the exercise intensity.

During ramp/incremental cycle exercise in study 3 (Chapter 5) participants reported a higher perception of exertion at 48 h, with an increase in reported RPE of 7% observed at the $\dot{V}O_2$ value of the pre-eccentric exercise GET. In studies 1 and 4 (Chapters 4 and 7) participants reported higher ratings of perceived exertion (RPE) during constant-load, severe intensity (70% Δ) cycle exercise 48 h after eccentric exercise, although RPE appeared to be unchanged during moderate (80% GET) exercise. It was of interest that the increased muscle pain and the elevated ventilatory response experienced by participants at 48 h did not significantly influence the perception of exertion during moderate intensity exercise. However, during severe intensity exercise, where the central, ventilatory cues may have played a more influential role in informing the perception of exertion, RPE was

significantly higher. In study 1 (Chapter 4) minute-by-minute differences in RPE values reported during severe exercise to volitional exhaustion before and 48 h after eccentric exercise were eliminated when expressed as a percentage of total exercise duration. This observation provided further evidence to support the proposition that there is a scalar-linear relationship between the rating of perceived exertion and exercise duration. Furthermore, differences in ventilation were also eliminated when expressed as a proportion of time to exhaustion. These findings suggest that there is a strong link between the ventilatory and perceived exertion responses to cycling following eccentric exercise.

8.1.4 Time to exhaustion

The perception of exertion may be considered fundamental to the individual exercise response when a participant is required to exercise to ‘volitional exhaustion’. Observations of elevated RPE have previously been associated with reduced time-trial performance in running and cycling following EIMD (Marcora & Bosio, 2007; Twist & Eston, 2009).

During incremental cycle exercise in study 2 (Chapter 5) and incremental knee extensor exercise in study 3 (Chapter 6) the observed decrease in time to volitional exhaustion and the associated reduction in peak work rate values were consistent with observations of impaired time trial performance brought about by the effects of eccentric exercise. Similarly, in studies 1 and 4 (Chapters 4 and 7) time to exhaustion was reduced following eccentric exercise, during constant load cycling at an intensity of 70% Δ . The precise nature of the accelerated fatigue development experienced with EIMD is poorly understood. Both central and peripheral fatigue factors have been implicated in the decreased endurance capacity observed following EIMD.

8.1.5 The metabolic response to dynamic exercise with EIMD

Central fatigue factors such as the enhanced production of inflammatory cytokines within regions of the brain responsible for movement, motivation, perception of effort and pain have been associated with decreased run time to fatigue (Carmichael et al., 2005). Similarly, peripheral fatigue factors originating within the damaged muscle tissue such as changes in metabolic function have been implicated in the decreased endurance capacity observed following EIMD (Asp et al., 1998)

In study 3 (Chapter 6), we used ^{31}P -magnetic resonance spectroscopy (^{31}P -MRS) to evaluate changes in muscle metabolism during dynamic incremental knee extensor exercise following eccentric exercise. The reduction in time to exhaustion following EIMD was not associated with an accelerated depletion of [PCr]. Time to exhaustion during the incremental knee extensor exercise was reduced by 12% following the muscle-damaging exercise. However the rate of [PCr] depletion was similar in the two experimental conditions with end exercise [PCr] remaining 13% higher 48 h after the muscle-damaging exercise. Increases in [Pi] and [Pi]:[PCr] were observed not only at rest but also during incremental exercise. However, the rate of increase in [Pi] was not altered as a result of the muscle damage however the premature termination of the test at 48 h resulted in there being no significant difference in end exercise [Pi] values. It was tempting to speculate that the limit to exercise tolerance was moderated by [Pi]. However wide inter-subject variability was observed with the [Pi] values, thus we could not reliably conclude that [Pi] was, indeed a limiting factor. Rather that end exercise [Pi] may have made an important contribution to the reduced time to exhaustion experienced following muscle damaging exercise.

8.1.6 Oxygen uptake kinetics and muscle oxygenation

While elevated [Pi] resultant to EIMD may contribute to decrements in performance of dynamic exercise, microvascular dysfunction observed following EIMD (Kano et al., 2005) may also contribute to impaired performance due to disruptions to delivery and distribution of O₂ within the capillary bed of the active muscle. We used Near Infrared Spectroscopy (NIRS) to assess muscle (haemoglobin + myoglobin) oxygenation and to determine the dynamic balance between oxygen delivery ($\dot{Q}O_2$) and oxygen uptake ($\dot{V}O_2$) following the onset of exercise. In particular, the deoxyhaemoglobin (HHb) concentration ([HHb]) NIRS signal was used to non-invasively estimate O₂ extraction in the skeletal muscle microcirculation.

The findings of study 4 (Chapter 7) demonstrated that EIMD resulted in a slowing of muscle deoxyhaemoglobin concentration [HHb] kinetics without altering pulmonary $\dot{V}O_2$ kinetics. The observation that the [HHb] kinetics were over 30% slower 48 h after the performance of eccentric exercise, indicated that there was an increase in the ratio of oxygen delivery to oxygen uptake ($\dot{Q}O_2:\dot{V}O_2$) during transitions to severe-intensity exercise. It was proposed that the elevated $\dot{Q}O_2:\dot{V}O_2$ ratio demonstrated by the slower [HHb] kinetic response (i.e. lower [HHb] at a given $\dot{V}O_2$ across the exercise transition) was due to compensatory changes to O₂ delivery. In summary, these data indicated that following EIMD, $\dot{V}O_2$ kinetics were preserved by compensatory increases in local muscle blood flow which were able to prevent a measurable slowing of $\dot{V}O_2$ kinetics.

8.2 Limitations

During studies 1 and 4 (Chapters 4 and 7) we employed constant-load, exhaustive exercise protocols in order to study the human response to dynamic exercise. The use of time or distance trials such as those employed by Marcora & Bosio (2007) or Twist and Eston (2009) may provide more ecologically valid models with which to study the effects of EIMD on the human response to dynamic exercise. However, transitions to constant load exercise provide the only appropriate platform for modelling of the kinetic response. Thus, whilst we appreciated the low ecological validity of using exhaustive, constant-load exercise protocols, we believed we were justified the need to employ our chosen exercise protocols in order to appropriately examine the human response to dynamic exercise with and without EIMD.

The relatively small sample sizes used for the investigations that comprise this thesis were approved as adequate to detect clinically or biologically worthwhile results by the Ethics Committee of the School of Sport and Health Sciences at the University of Exeter following appropriate, rigorous power calculations (See Appendix A).

All participants were recreationally active, healthy young men and as such interpretation of our findings should be restricted to matching populations. Extrapolating findings to other populations such as highly trained or sedentary groups would be inappropriate. The protective adaptation to a single bout of eccentric exercise, the ‘repeated bout effect’ (Nosaka & Clarkson, 1995) may last up to 6 months for most symptoms of EIMD (Nosaka et al., 2001a). Thus the training status of participants may have a profound influence on their response to dynamic exercise with EIMD.

8.3 Implications and future directions

The main findings of the studies that comprise this thesis have implications for the interpretation of human responses to physical exercise following eccentric exercise. An awareness of potential changes in metabolic, ventilatory, gas exchange and perceived exertion responses following eccentric exercise may help coaches, exercise scientists and health and fitness practitioners to make more informed decisions regarding advice given to their charges. As has been alluded to earlier in this chapter, the use of constant-load, exhaustive exercise protocols is not ecologically valid. In future, researchers should seek to investigate changes in the human response following EIMD using, for example, distance or time trial protocols, such as those used by Marcora and Bosio (2007) and Twist and Eston (2009). The increased popularity of high intensity exercise training methods including resistance training and plyometrics may lead to an increased incidence of EIMD in the active population. Recreationally active individuals should be aware of the potential alterations to their performance capacity in the days following this type of training. Future research should also attempt to elucidate differences in the human response to EIMD between individuals of different training and activity status.

The influence of eccentric, muscle damaging exercise on the human response to dynamic exercise has only become the focus of scientific investigation in more recent years (see section 2. 7). Whilst this thesis has extended the examination of some of these responses, many questions remain unanswered. The various human responses to dynamic exercise evaluated within this thesis; including ventilatory, gas exchange, perceived exertion and metabolic responses have only been observed 48 h after EIMD. The use of a discrete observational period was intentional with the time of 48 h chosen to correspond with the period of maximal muscle

soreness. However, future research should attempt to evaluate time-course changes in these and other human responses during the hours and days following eccentric, muscle damaging exercise in order to elucidate any potential mechanistic links with time-course changes in known markers of EIMD such as the loss of force generating capacity.

In studies 3 and 4 (chapters 6 and 7) we used animal studies (Carmichael et al., 2005 and Kano et al., 2005, chapters 6 and 7 respectively) to inform our understanding and provide the basis of the research question in human subjects. Understandably, we have not been able to use some of the techniques employed with animal models. Carmichael et al. (2005) dissected the brains of eccentrically exercised mice to reveal that increases in inflammatory cytokine concentrations in the cortex and cerebellum were associated with reduced exercise tolerance. Inspired by the invasive intravital microscopy studies of rat *spinothrapezius* muscle by Kano et al. (2005), we used NIRS to facilitate the non-invasive assessment of muscle oxygenation following EIMD. In study 4 (chapter 7) we observed a reduced [HHb] signal in the *vastus medialis* muscle for a given $\dot{V}O_2$ at exercise onset 48 h during cycling after squatting exercise. Future research could extend the use of NIRS to facilitate the non-invasive assessment of muscle oxygenation following EIMD by examining the response to a range of different damage and exercise protocols.

8.4 Conclusion

In conclusion, the studies that comprise this thesis have demonstrated how the human response to dynamic exercise may be altered following exercise-induced muscle damage. The structural and functional changes that characterise EIMD have been demonstrated to generate additional or altered ventilatory stimuli during exercise such that the ventilatory

response to cycle exercise was elevated at exercise intensities above and below the gas exchange threshold. Furthermore, the augmented ventilatory response observed has been shown to result in the gas exchange threshold occurring at a lower work rate without a concomitant change in the lactate threshold. In combination, these findings demonstrate that the enhanced ventilation experienced following EIMD may be controlled by additional or altered neurogenic stimuli rather than changes in metabolic factors. Increases in the ventilatory response were associated with elevated ratings of perceived exertion (RPE) and a reduction in time to exhaustion. Differences in minute-by-minute ventilation and RPE values were eliminated when expressed as a percentage of total exercise duration. These findings indicate that there is a strong link between the ventilatory and perceived exertion responses to cycling following EIMD. Although we have shown that an increase in blood [La] is not an obligatory consequence of EIMD, we have demonstrated, using ^{31}P -MRS, that muscle metabolic response to dynamic exercise is altered following EIMD. The accelerated fatigue observed following EIMD may be related either to the increased [Pi] that was observed at rest and throughout incremental exercise, or to other unmeasured peripheral or central factors. Finally, we showed that peripheral microvascular dysfunction resultant to EIMD may contribute to impaired performance due to disruptions to delivery and distribution of O_2 within the capillary bed of the active muscle. Using Near Infrared Spectroscopy (NIRS) we demonstrated that EIMD resulted in a slowing of muscle deoxyhaemoglobin concentration [HHb] kinetics without altering pulmonary $\dot{\text{V}}\text{O}_2$ kinetics. Findings demonstrated that there was an increase in the ratio of oxygen delivery to oxygen uptake ($\dot{\text{Q}}\text{O}_2 : \dot{\text{V}}\text{O}_2$) following EIMD which was due to compensatory changes to O_2 delivery.

CHAPTER 9

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CHAPTER 10

APPENDICES

APPENDIX A: EXEMPLAR APPLICATION FOR ETHICAL APPROVAL

1. Project title:

The metabolic response to exhaustive exercise in men with and without exercise-induced muscle damage

2. Purpose of project and its academic rationale:

The aim of this study is to investigate the influence of eccentric, muscle-damaging exercise on 1) muscle metabolism during exercise to exhaustion using a whole-body magnetic resonance spectrometer (MRS) and 2) metabolic thresholds during ramp exercise to exhaustion on a cycle ergometer. We hope to reveal any link between changes in Pi/PCr and pH and the limitations to exercise tolerance in humans with and without exercise-induced muscle damage (EIMD). In addition we hope to reveal any shift in metabolic thresholds which might contribute to limiting exercise tolerance following EIMD.

EIMD has a profound impact on muscle structure and function. Structural changes include sarcomere disruption and damage to t-tubules, sarcoplasmic reticulum and sarcolemma (Fridén & Lieber, 2001). This disruption leads to increased influx of extracellular Ca^{2+} into the sarcoplasm and an efflux of intramyocyte contents such as creatine kinase and myoglobin into the bloodstream (Warren et al., 1999). These degenerative changes are associated with delayed onset muscle soreness (DOMS), a reduction in maximal force generating capacity (Clarkson et al., 1992) and a shorter time to exhaustion (Carmichael et al., 2006). Changes in metabolic function include impaired muscle glycogen resynthesis, significantly lower resting muscle glycogen content (Asp et al., 1998) and an elevated blood lactate response (Gleeson et al 1998). It has been suggested that these changes reflect a shift in the muscle metabolic profile to an increased reliance on non-oxidative metabolism following EIMD (Tee et al., 2007).

Magnetic resonance spectroscopy (MRS) can be used to measure parameters of muscle metabolic function including the dynamic changes in the ratio of inorganic phosphate to phosphocreatine (Pi/PCr) and intracellular pH. To our knowledge, no previous study has investigated the metabolic response to dynamic exercise following EIMD using MRS.

Cycle exercise to exhaustion using a ramp protocol is commonly used to determine the gas exchange threshold (GET) and VO_{2max} and to set subsequent exercise or training intensities. Although VO_{2max} is unchanged, the blood lactate response to incremental exercise is elevated following EIMD (Gleeson et al., 1998). To our knowledge, no previous study has investigated the effect of EIMD on potential alterations to GET following EIMD.

This research will enhance our understanding of the link between metabolic changes and the limit to exercise tolerance in humans with and without EIMD. Any changes in muscle metabolic responses resulting from eccentric exercise could have important implications for athletes using plyometric or resistance training.

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3. Description of all proposed methods and measurements:

Methods

Experimental Overview

Participants will be asked to complete an eccentric exercise protocol, designed to induce muscle-damage. Before and 48 h after the eccentric exercise, the participants will complete two incremental exercise tests to exhaustion. Specifically, participants will complete a single-legged, knee extension test inside a whole body MRS (Philips Gyroscan Clinical Intera). Following ~2 h rest, markers of muscle damage will be measured before participants complete a ramp exercise test on an electronically braked cycle ergometer (Lode Excalibur Sport).

Single-legged, knee-extension test

The single-legged, knee-extension exercise tests will be conducted in the prone position with the participants positioned inside a whole body MRI system. A 6-cm 31P transmit-receive surface coil will be placed within the participants bed, and the participants will lie with the coil centred over the quadriceps muscle of the leg to be exercised. Participants will then be secured to the ergometer bed with Velcro straps at the thigh, buttocks, and lower back to minimize extraneous movement during the protocol. The foot of the leg to be exercised will be connected to a pulley system that permits a nonmagnetic weight to be lifted and lowered and work rate to be calculated. An initial basket load of 0.5 kg will be increased by 0.5 kg at the end of each minute until the participants are no longer able to maintain the kicking frequency. Exercise will be performed at a rate of 40 reps/min with the participants lifting and lowering the mass over a distance of ~ 0.22 m in accordance with a visual cue projected onto the front wall of the scanner room. Participants will receive strong verbal encouragement to continue for as long as possible while maintaining appropriate form. The 31p-MRS interrogation of the quadriceps will coincide with the contraction phase of the knee extensors.

Cycle exercise test

Following ~2 h rest participants will be asked to complete an incremental exercise test to volitional exhaustion on an electronically braked cycle ergometer. Following 3 min of unloaded baseline cycling, the work rate will be increased in a ramp fashion (30Wmin^{-1}) until the participant is unable to continue. Participants will receive strong verbal encouragement to continue for as long as possible. The participants will cycle at a self-selected pedal rate (between 70 and 90 rpm) and this pedal rate along with the saddle and handlebar heights will be recorded and reproduced in subsequent tests. Cycling cadence must be replicated in the post eccentric exercise condition. Any change in cadence that may result from exercising in the damaged condition would influence fibre type recruitment and consequent substrate usage and would thus confound the results.

Eccentric, muscle-damaging exercise protocol

Participants will perform 100 (smith) squats, as 10 sets of 10 repetitions. The load on the bar will correspond to 70% of each participant's body mass. During the movement the bar will be positioned on the participant's shoulders. The participant's head must be kept forward, the back straight and the feet flat on the floor, toes pointing forward, with equal distribution of weight through fore-foot and heel. Before the bar is disengaged it will be positioned on the back of the shoulders and grasped to the sides. Feet will be positioned under the bar, with the back erect and legs fully extended (knee = 180°). One complete squat comprises two phases. The descent phase involves eccentric (muscle damaging) action of the knee extensors to lower the bar to a knee angle of just past 90°. The lifting phase involves concentric action to return the bar to the starting position. Participants will be provided with the ACSM current comment on the safety of squat exercise and trained in safe procedures. Participants will be allowed a much time as required to recover between sets to facilitate their completion of the protocol. Participants will be made aware that if they are unable to complete the full 100 squats; their data may not be included.

Measurements

Markers of muscle damage

All markers of muscle damage will be measured in the order listed below, before, immediately after and 24 and 48 h after the muscle damaging protocol.

Plasma Creatine kinase (CK)

Plasma CK activity will be assessed from fingertip capillary samples. Approximately 300µl of blood will be centrifuged to provide two 20µl samples of plasma. Each sample will be added to 1ml of a composition of reagents supplied by Randox (CK-NAC 110, Randox Laboratories Ltd., Crumlin, Co. Antrim, UK) in a semi-micro cuvette and mixed on a vortex mixer (Jencons vortex mixer, Jencons PLC, UK). Following 1min incubation at 37°C and during continued incubation, absorbance of 340 nm light will be recorded in a spectrophotometer (Jenway 6310 spectrophotometer, Jenway, Essex, UK) at 0, 1, 2 and 3min. CK values will be calculated using the formula $CK(U/l) = 8095 \times \Delta \text{ absorbance } 340 \text{ nm/min}$.

Perceived muscle soreness

Soreness of the knee extensors will be assessed using a 10-point visual analogue scale (VAS). The participants will be instructed to perform a squat with hands on hips, squatting to a knee angle of ~90° and rate their perception of soreness by placing a mark on the scale.

Isokinetic Peak Torque – knee extensors

Peak knee extensor torque will be measured using a Biodex B-2000 isokinetic dynamometer (Biodex Corp, Shirley, NY,) at both fast (180 deg.s⁻¹) and slow (30 deg.s⁻¹) velocities. Testing will be preceded by a standardised warm-up of 2 minutes cycling at 50 W on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands followed by static stretching exercises of the knee extensor/flexor muscle groups. The participant will be positioned in an upright position with the knee and hip of the test limb fixed at 90° (full knee extension = 180°) and 100° (full hip extension = 180°) respectively. The total range of motion for each participant will be manually determined by the investigator, while the mass of the limb will be recorded by the dynamometer to enable gravitational correction of peak torque values.

The highest value of five maximal voluntary contractions (MVCs) at slow (0.52 rad, 30 deg.s⁻¹) and then fast (3.14 rad, 180 deg.s⁻¹) angular velocities will be recorded. One minute of rest will be allowed between each angular velocity with the slower angular velocity always preceding the faster angular velocity during the protocol. Visual feedback, displaying real time force, will be used to encourage maximal efforts. Participants will also be consistently encouraged to exceed target values, based upon those achieved during familiarisation.

During Single-legged, knee-extension

All measures will be taken before and 48 h after the muscle damaging protocol.

MRS measurements.

MRS will be performed in the Peninsula Magnetic Resonance Research Centre using a 1.5-T superconducting magnetic resonance scanner (Philips Gyroscan Clinical Intera). The muscle will be positioned relative to the coil before the signal is optimised via a number of pre-acquisition steps. Following this, an automatic shimming protocol will be undertaken and matching and tuning of the coil will be performed. The participants will be visually queued via a display consisting of two vertical bars, one that moves at a constant rate with a frequency of 0.67 Hz and one that monitors foot movements via a sensor present within the pulley to which they are connected. The work done by the participants will be recorded via a nonmagnetic strain gauge present within the pulley mechanism. Before exercise, during exercise, and during recovery, data will be acquired every 1.5 s, with a spectral width of 1,500 Hz and 1,000 data points. Phase cycling with four phase cycles will be employed, leading to a spectra being acquired every 6 s. The subsequent spectra will be quantified via peak fitting, assuming prior knowledge, using the jMRUI (version 2) software package and the AMARES fitting algorithm.

Near Infra-Red Spectroscopy (NIRS)

The extent to which muscle damage alters muscle oxygenation and oxygen extraction will be measured during the knee-extension exercise using a commercially available NIRS system (NIRO-300). The probe will be secured 10-12 cm above the right knee joint, with location marked during the first test to enable reproduction of the probe position in the test at 48 h after eccentric exercise.

During cycle exercise

All measures will be taken before and 48 h after the muscle damaging protocol.

Pulmonary gas exchange

Throughout the cycle exercise tests pulmonary gas exchange will be measured breath by breath via an online gas analysis system (Cortex MetaLyzer II). Gas exchange will be recorded throughout the tests via the Cortex Metasoft 3.1 software. The system will be calibrated prior to every test in accordance with manufacturer's guidelines against known concentrations of cylinder gases and a three litre calibration syringe (for flow volume).

Heart rate

Throughout the cycle exercise tests heart rates will be monitored using a wireless chest strap telemetry system (Polar Electro) and will be recorded continuously via a link to the Cortex gas analysis system.

Blood lactate

Finger-tip blood samples will be collected into a capillary tube immediately before and after and at 1-min intervals during each cycle exercise test. Samples will subsequently be analyzed for whole blood lactate (YSI 2300 Sport, Yellow Springs, Ohio, USA).

Ratings of Perceived Exertion (RPE)

Participants will be familiarised with the Borg 6-20 RPE scale and provided with standardised instructions on how to employ the scale. They will be asked to report an RPE value at 1-min intervals throughout the test.

Costings

The Research Committee has approved the use of the Peninsula MRS for 18 1-hour sessions. In addition, analysis of 48 finger-tip blood samples for the analysis of plasma CK activity and approximately 300 finger-tip blood samples for the analysis of blood lactate concentration will need to be funded. The cost of blood analysis will be covered by Dr Rowlands' research fund.

4. Participants: Recruitment methods, number, age, gender, exclusion/inclusion criteria.

~9 healthy, physically active males who have not participated in any resistance training of the lower limbs prior to assessment will be asked to volunteer for the study. The use of male subjects is to facilitate comparison with previous investigations undertaken during this PhD as well as previous investigations into exhaustive exercise in the MRS. No payment or reward will be offered to participants. All individuals will be asymptomatic of illness, disease and pre-existing injuries and will be able to exercise to exhaustion. Participants will be excluded if they have a personal history of diabetes, hypertension or vascular disease. The School of Sport and Health Sciences 'safety guidance sheet for health screening of subjects prior to exercise testing' will be referred to prior to each exercise test. Participants will provide written informed consent before beginning the study. The health status of each participant will be ascertained by a Physical Activity Readiness Questionnaire (PAR-Q) before each exercise test (as recommended by the School of Sport and Health Sciences).

Sample size: Using the MRS, the primary outcome variable is the PCr kinetic response as measured by MRS. No previous studies have investigated the influence of EIMD on muscle metabolism during knee extensor exercise to exhaustion using an MRS. However, Lund et al. (1998) have reported reductions in the resting PCr/Pi ratio of 31% following EIMD. Assuming a combined standard deviation of 20% a sample size of 9 would detect a 31% decrement at a power of 0.8 and alpha of 0.05. During ramp exercise on a cycle ergometer, the primary outcome variable is the GET. No previous studies have investigated the influence of EIMD on changes in GET. However, Gleeson et al. (1998) observed an elevated blood lactate response to incremental cycle exercise following EIMD (10.9 increasing to 12.6 mmol.l⁻¹). Assuming a combined standard deviation of 1.2 mmol.l⁻¹ a sample size of 9 would detect a 1.7 mmol.l⁻¹ increase at a power of 0.8 and alpha of 0.05.

5. Consent and participant information arrangements, debriefing.

Please find attached the intended information letters and consent forms.

6. A clear but concise statement of any specific ethical considerations raised by the project and how you intend to deal with them.

Please find attached detail of the eccentric, muscle-damaging protocol.

Although the intention is to provoke a specific 'muscle-damage' response which causes discomfort for up to 4 days, it is important to ensure that no other injury is caused. With this in mind, participants will be screened to ensure that they are free from pre-existing back and knee injuries. They will be instructed in the correct lifting technique and supported by experienced spotter throughout. Sufficient recovery between sets will be allowed to minimise impact of fatigue on technique.

All participants will experience muscle pain which will peak 24 -48 h following the eccentric exercise protocol. Participants will be made fully aware that they will experience pain and that they will be asked to exercise maximally whilst experiencing pain.

We intend to pay due consideration to the following risk assessments and safety guidance

SSHA/HAZ/0002	Exercise Testing Volunteer Subjects in Laboratory SSHS General Health Questionnaire Current Health Status Questionnaire
SSHS/HAZ/0010	Use of Magnetic Resonance Techniques to Obtain Anatomical and Functional Information About the Body
SSHA/HAZ/0003	Measurement of Oxy- and Deoxyhaemoglobin Using Near Infrared Spectroscopy.
SSHA/HAZ/0007	Muscle Strength Measurement Using Biodex System 3
SSHA/HAZ/00026	Muscle Damaging Squat Exercise Using a Smith Machine
SSHS/COSHH/0005	Collection of Capillary Blood Samples

Muscle damaging protocol (Squats)

The participants will perform 100 (smith) squats, to be performed as 10 sets of 10 repetitions. The load on the bar will correspond to 70% of each participant's body mass.

During the movement the bar will be positioned on the participant's shoulders. The participant's head must be kept forward, the back straight and the feet flat on the floor, toes pointing forward, with equal distribution of weight through fore-foot and heel.

Before the bar is disengaged it will be positioned on the back of the shoulders and grasped to the sides. Feet will be positioned under the bar, with the back erect and legs fully extended (knee = 180°).

One complete squat comprises two phases. The descent phase involves eccentric action of the knee extensors to lower the bar to a knee angle of just past 90°. The lifting phase involves concentric action to return the bar to the starting position.



- Participants will be free of pre-existing knee and or back injuries.
- Participants will be fully familiarised with the machine and the lifting techniques prior to commencement of the protocol.
- Experimenter to ensure that the correct lifting and lowering technique is employed at all times.
- The spotter catch system must be set to prevent the bar from pinning the subject at full flexion.
- Recovery of 1 min between sets must be taken to minimise the impact of fatigue on technique.

Participants will experience an immediate and prolonged reduction in muscle function, most notably a reduction in force-generating capacity as a result of performing the squats. Leg muscles may be pain-free for approximately 8 hours but soreness will increase and peak over the next 24–48 hours. All discomfort usually subsides within 96 hours (4 days).

APPENDIX B: EXEMPLAR PARTICIPANT INFORMATION SHEET



SCHOOL OF SPORT AND
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Exercise-induced muscle damage and exercise in men

INFORMATION SHEET FOR PARTICIPANTS

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to take part. If you decide to take part we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the Aim of the Project?

The aim of the proposed study is to investigate the influence of muscle-damaging exercise (squats) on muscle responses during exercise to exhaustion. We intend to measure responses during knee exercise in a whole-body magnetic resonance spectrometer (scanner) and during cycle exercise.

This research will improve our understanding of the link between changes in muscle after squats and the limit to exercise tolerance in humans. Any changes in muscle responses resulting from squats could have important implications for athletes using plyometric or resistance training.

What Type of Participants are Needed?

We are looking for 9 participants who are physically active, free from back and knee injuries and have not done any resistance training of the legs for at least 6 months.

What will Participants be Asked to Do?

If you agree to take part in this project, you will be asked to come to the Richards Building Physiology Laboratory at the School of Sport & Health Sciences on five separate occasions to take part in exercise tests as follows:

1. During the first visit to the laboratory we will measure your height and weight. You will then be fully familiarised with the equipment and the exercise tests to be used during the study. This will include a practice session in a 'mock scanner'.
2. During your second visit you will be asked to complete a knee exercise test inside the scanner. After a rest of about 2 hours you will be asked to complete an exercise test on a cycle ergometer when you will be asked to breathe through a mouthpiece. Both tests are incremental and will start off gently but will get harder and harder. You will be asked to exercise until you are unable to continue.

Before the cycle tests you will be asked to provide a finger-tip blood sample. You will be asked to assess how sore your legs feel by placing a mark on a visual scale and to perform a maximal knee extension test to measure the strength of your thigh muscles.

3. On your third visit you will be asked to perform 100 squats, as 10 sets of 10 repetitions, in order to provoke muscle damage. The load on the bar will be set to 70% of your body mass. You will be given as much time as you need to recover between sets but if you cannot complete the 100 squats your exercise test results may not be used.

Following this, you will be asked to provide a finger-tip blood sample, assess how sore your legs feel and perform a maximal knee extension test.

You will experience an immediate loss of muscle strength as a result of performing the squats. Your legs may be pain-free for about 8 hours but soreness will increase and peak over the next 24–48 hours. The pain you will feel will vary from person to person but you may struggle with some daily tasks such as walking downstairs. All discomfort usually subsides within 4 days.

4. On your fourth visit (24 h after the squats) you will be asked to provide a finger-tip blood sample, assess how sore your legs feel and to perform a maximal knee extension test.
5. On your fifth visit (48 h after the squats) you will be asked to repeat the exercise test and measures explained in 2 above.

Can Participants Change their Mind and Withdraw from the Project?

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

What Data or Information will be Collected and What Use will be Made of it?

Your body height and weight will be measured. Finger-tip blood samples will be analysed for blood lactate and plasma creatine kinase activity. Measures of muscle soreness and strength will also be taken. During cycle exercise, gas exchange, heart rate and ratings of perceived exertion will be recorded. During exercise in the scanner, changes in the muscle will be measured by the scanner and surface electrodes will take recordings using near-infrared spectroscopy (NIRS) to measure muscle oxygen status. Results of this project may be published but any data included will in no way be linked to any specific participant. You are most welcome to request a copy of the results of the project should you wish. The data collected will be retained indefinitely and securely stored in such a way that only those mentioned above will be able to gain access to it.

What if Participants have any Questions?

If you have any questions about our project, either now or in the future, please feel free to contact:-

Dr. Ann Rowlands

University Telephone Number: 01392 262878;

email: a.v.rowlands@exeter.ac.uk

Rosemary Davies

email: rd217@exeter.ac.uk

The Ethics Committee of the School of Sport and Health Sciences has reviewed and approved this project

APPENDIX C: EXEMPLAR PARTICIPANT CONSENT FORM



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Exercise-induced muscle damage and exercise in men

CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:-

1. I will be asked to perform two incremental knee-extension exercise tests to exhaustion inside a whole body magnetic resonance spectrometer (scanner);
2. I will be asked to perform two incremental cycle exercise tests to exhaustion on a cycle ergometer;
3. I will be asked to perform muscle-damaging exercise, involving 100 squats; I will be given as much time as I need to recover between sets but if I cannot complete the 100 squats my exercise test results may not be used;
4. I will experience pain for several days following the muscle-damaging exercise which may mean that I will struggle with some daily tasks such as walking downstairs and that I will be expected to exercise during this period;
5. I will be asked to provide finger-tip blood samples;
6. I will be asked to assess my muscle soreness and to perform maximal knee extensions;
7. my participation in the project is entirely voluntary;
8. I am free to withdraw from the project at any time without any disadvantage;
9. any raw data on which the results of the project depend will be retained in secure storage;
10. the results of the project may be published but my anonymity will be preserved.

I agree to take part in this project.

.....(Signature of participant).....(Date)

APPENDIX D: EXAMPLAR ETHICAL APPROVAL



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Certificate of Ethical Approval

Proposal 6 (22/10/08)

Title: The metabolic response to exhaustive exercise with and without exercise-induced muscle damage

Applicant: Dr Ann Rowlands (Lecturer) with Prof Roger Eston, Prof Andy Jones, Dr Daryl Wilkerson, Dr John Fulford and Ms Rosey Davies (Research Student)

The proposal (circulated previously) was discussed by the Committee. The Committee advised that the application should be amended as follows:

- i. Clarify what the procedure would be if someone could not complete 100 squats and make people aware on the Information Sheet that they might be excluded if they cannot complete the protocol.
- ii. Include men in the title of the project.
- iii. The cadence should not be pre-determined because cadence influences fibre type recruitment which influences substrate use.

Decision: the Committee AGREED to provisionally approve the proposal until July 2009, but required the amendments outlined above (i-iii). The amendments need to be returned to AH for approval by JW prior to the commencement of the study.

Decision: The amendments (as outlined above) were received and were deemed satisfactory and the proposal was approved.

School Ethics Committee Reference Number: 22/10/08#6

Signature: *Joanne R Welsman*



Date: *12/11/08*

Name/Title of Chair: Dr J Welsman

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

APPENDIX E: EXEMPLAR RISK ANALYSIS; SQUATTING PROTOCOL

SECTION ONE ADMINISTRATIVE DETAILS

REFERENCE: SSHA/HAZ/0026	SIGNATURE OF ASSESSOR: 
DEPARTMENT: Sport & Health Science	SIGNATURE OF HEAD OF DEPT: 
DATE: 2-11-2006	ASSESSED UNDER OTHER REGULATIONS? YES <input type="checkbox"/> NO <input checked="" type="checkbox"/>
REMEDIAL ACTION REQUIRED? YES <input type="checkbox"/> NO <input checked="" type="checkbox"/>	OTHER ASSESSMENT REFERENCE:
REMEDIAL ACTION PRIORITY? HIGH <input type="checkbox"/> MEDIUM <input type="checkbox"/> LOW <input type="checkbox"/>	

WORK ACTIVITY: <i>Muscle damaging squat exercise using a Smith machine</i>	
BRIEF DESCRIPTION: <i>The squat is a barbell exercise where the individual starts in a standing position with the barbell on the back, and bends the knees to squat down until the thighs are parallel with the floor. The Smith machine is a linear exercise machine which enables squats to be performed more safely due to the vertical tracking bars and spotter safety catch system. 10reps x 10 sets at 70% body mass squats are performed in this muscle damaging protocol.</i>	
ESTIMATED No OF EMPLOYEES AT RISK: N/A	ESTIMATED No OF NON EMPLOYEES AT RISK: N/A

SECTION TWO HAZARD IDENTIFICATION

HAZARD = something with the potential to cause harm * Identify HAZARDS, circle KEYWORDS

HAZARDS	KEYWORDS
Physical	
Confined space	asphyxiant cold hot toxic irritant lone working ventilation
Construction	CONDAM Regs Ass't scaffolding work at height falling object
Display Screen Equip't	DSE Regs Ass't desk chair electricity eyestrain eye test posture
Electricity	PAT testing live static induced arc heat burn shock 240V AC 405V AC high voltage
Environment	temperature humidity light sound space
Fire	flammable combustible explosion oxygen heat
Handling	MHO Regs Ass't abrasive heavy lifting pushing pulling sharp hot cold awkward
Heat / Cold	radiation conduction convection burn scalds touch
Housekeeping	falling tripping slipping storage space cables combustion sources hygiene
Machinery	MHO Regs Ass't cutting rotating sliding falling entrapment breakage ejection of parts electricity radiation heat cold
Movement	slip fall trip wet ice steps stairs height
Pressure / Vacuum	burst release lines joints container cylinder explosion leak blockage relief/control failure
Radiation (Ionising)	radioisotope X-ray alpha beta gamma contamination exposure use storage disposal
Radiation (Non Ionising)	ultra-violet infrared laser microwave burns welding eye cataract
Transport	road markings road signs dangerous loads minibus forklift truck trolley truck commercial vehicle passenger lift goods lift footpath ramp car boat
Water	diving drowning slipping electricity
Weather	hot cold wet ice wind lone-working frostbite heat-stroke sunburn skin cancer hypothermia
Chemical	
Physical state	solid dust liquid gas vapour fume hot cold
Properties	COSHH Ass't toxic corrosive irritant carcinogen allergen flammable unstable explosive
Routes of Entry	inhalation ingestion skin contact
Biological	
Type	COSHH Ass't microorganism bacteria virus parasites cell culture storage disposal
Properties	infectious pathogenic carcinogenic mutagenic teratogenic storage disposal
Genetic modification	GMO Regs Ass't storage disposal
Psychological	
Type	fatigue stress trauma
Other hazard(s): keywords: <u>Knee and back injury</u>	

PTO

SECTION THREE RISK ASSESSMENT

RISK = a combination of the likelihood a hazard will cause injury and the severity of the injury

* Quantify risk for each hazard identified using the following table:

Likelihood of injury	Score A	Severity of injury	Score B
improbable	1	very minor injury; abrasions / contusions	1
remote	2	minor injuries; cuts / burns	2
possible	3	major injuries; fractures / cuts / burns / damage to internal organs	3
probable	4	severe injury; amputation / eye loss / permanent disability	4
likely	5	death	5

- Enter Hazards identified in Section 1
- Enter Existing control measures
- Quantify Risk factor by multiplying Score A and Score B, taking account of existing control measures.
- If Risk factor is over 5: take Remedial Action to improve Existing control measures or abandon the task
- If Risk factor is 5 or under, the risks are under adequate control, but should be carefully monitored

Hazards	Existing control measures	Score A	Score B	Risk (A x B)	Remedial Action
Back and Knee Injury	<ul style="list-style-type: none"> • Subjects must be free of existing knee and/or back injuries. • Use smith machine rather than free weights as the spotter catch system prevents the bar from pinning the subject at full flexion and the parallel tracking bars enable greater straight-line stability. • Ensure the subject is familiar with and uses the correct lifting and lowering technique at all times inc. keeping a straight back, not exceeding a depth of 90° at knee • Allow sufficient recovery between sets to minimise impact of fatigue on technique. 	1	3	3	

APPENDIX F: THE BORG (6-20) RPE SCALE (slightly modified)

6 No exertion at all

7

Extremely light (7.5)

8

9 Very light

10

11 Light

12

13 Somewhat hard

14

15 Hard (heavy)

16

17 Very hard

18

19 Extremely hard

20 Maximal exertion

Borg RPE scale (Borg, 1998)

Instructions on how to use the Borg Rating of Perceived Exertion (RPE) Scale

While exercising, we want you to rate your perception of exertion. This feeling should reflect how heavy and strenuous the exercise feels to you, combining all sensations and feelings of physical stress, effort, and fatigue. Do not concern yourself with any one factor such as leg pain or shortness of breath, but try to focus on your total feeling of exertion.

Look at the rating scale below while you are engaging in an activity; it ranges from 6 to 20, where 6 means "no exertion at all" and 20 means "maximal exertion." When you are asked to do so, choose the number from below that best describes your level of exertion. This will give you a good idea of the intensity level of your activity.

Try to appraise your feeling of exertion as honestly as possible, without thinking about what the actual physical load is. Your own feeling of effort and exertion is important, not how it compares to other people's. Look at the scales and the expressions and then give a number.

9 corresponds to "very light" exercise. For a healthy person, it is like walking slowly at his or her own pace for some minutes

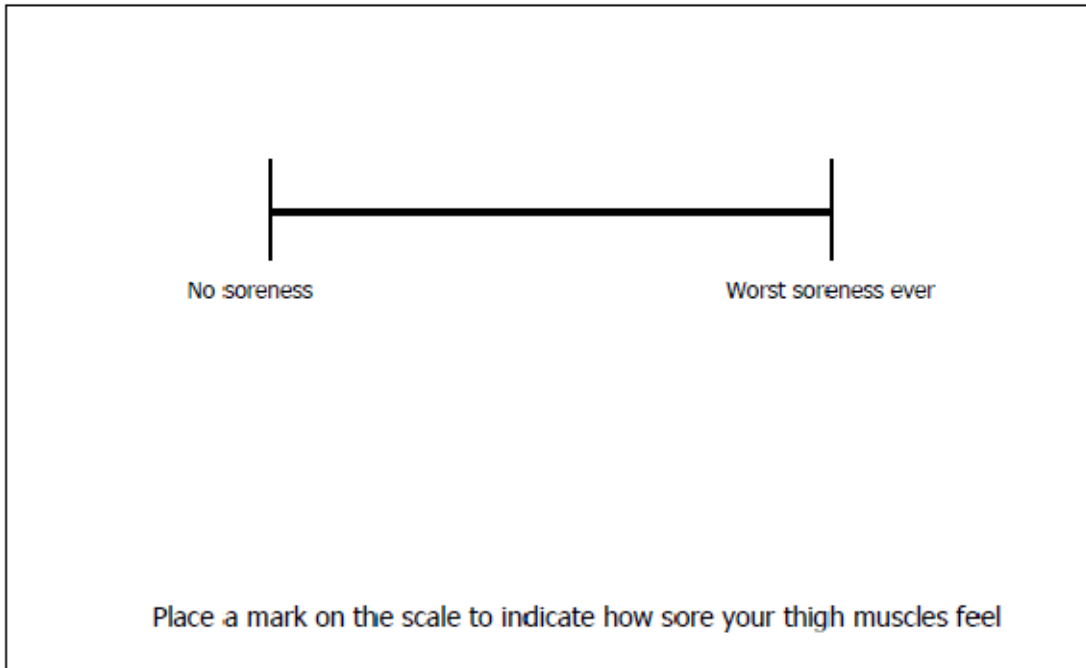
13 on the scale is "somewhat hard" exercise, but it still feels OK to continue.

17 "very hard" is very strenuous. A healthy person can still go on, but he or she really has to push him- or herself. It feels very heavy, and the person is very tired.

19 on the scale is an extremely strenuous exercise level. For most people this is the most strenuous exercise they have ever experienced.

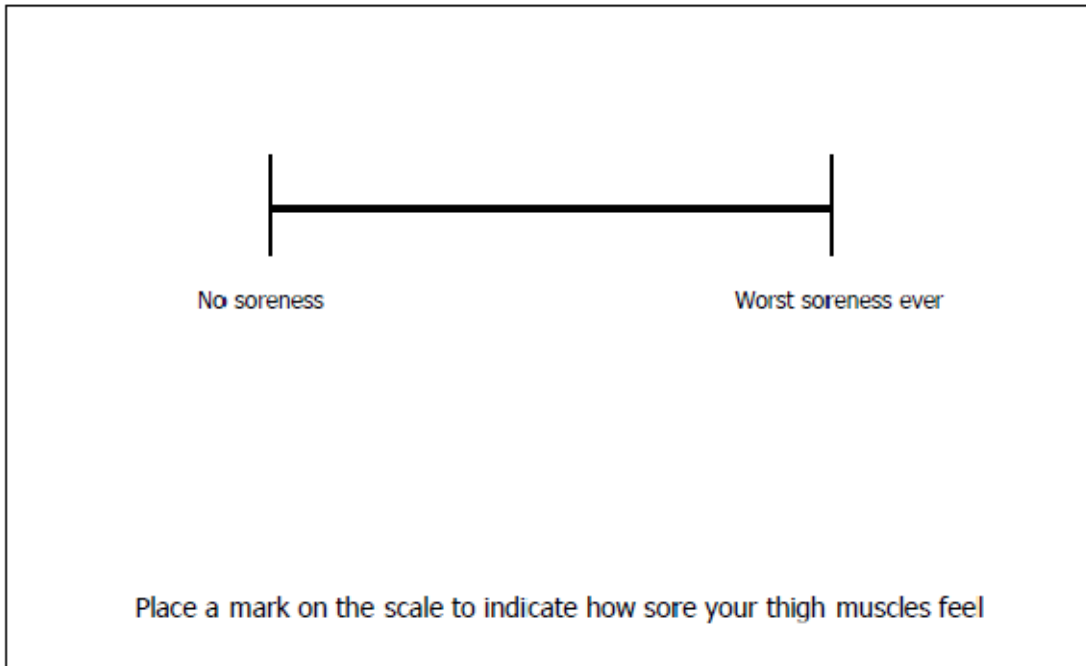
Adapted from Borg (1998)

APPENDIX G: VISUAL ANALOGUE SCALE (VAS)



A horizontal line with vertical tick marks at each end. The left tick mark is labeled "No soreness" and the right tick mark is labeled "Worst soreness ever".

Place a mark on the scale to indicate how sore your thigh muscles feel



A horizontal line with vertical tick marks at each end. The left tick mark is labeled "No soreness" and the right tick mark is labeled "Worst soreness ever".

Place a mark on the scale to indicate how sore your thigh muscles feel

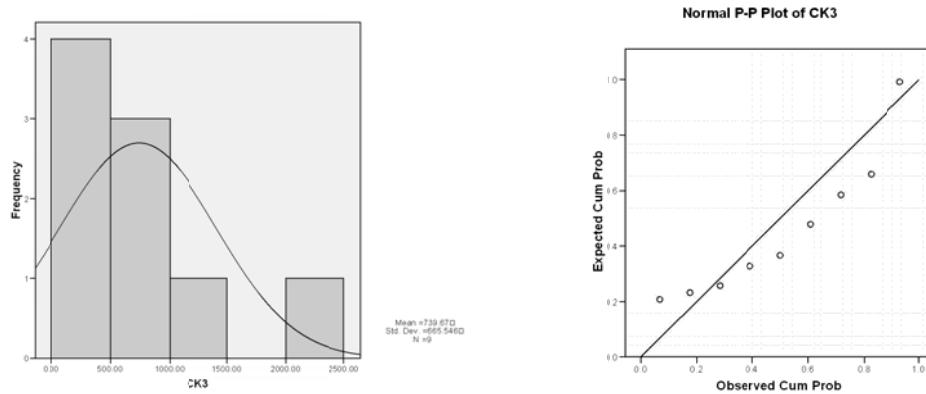
APPENDIX H: STATISTICAL ASSUMPTIONS

In each of the four studies included in this thesis, all data were checked for assumptions of parametric data prior to analysis. In addition to ensuring that all data were measured at the interval level or above and that all scores were independent, all data were checked for normality of distribution and homogeneity of variance.

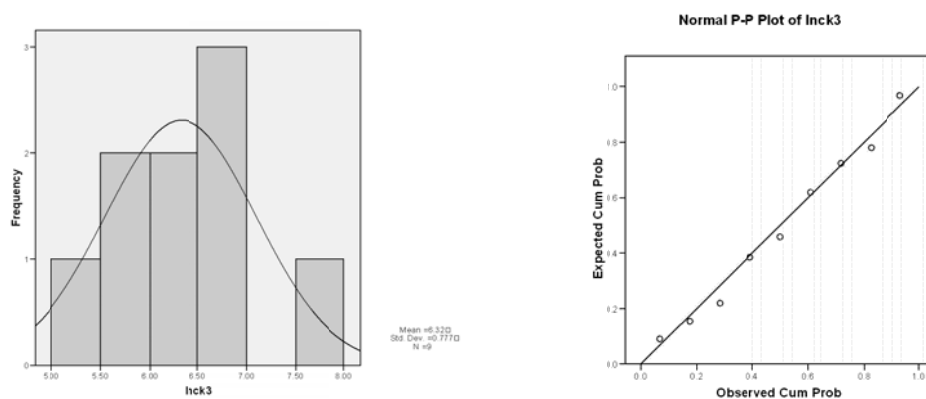
Normal distribution

The assumption of normally distributed data was checked using histograms, cumulative probability plots and the Shapiro-Wilk test. All data were normally distributed with the exception of Creatine kinase (CK) data. CK data was therefore log-transformed to ensure normal distribution before any analyses were carried out. Below is an example of the frequency distribution curve and cumulative probability plot for A: raw CK data (not normally distributed) and B: log transformed data (normally distributed).

A



B



Below is an example of an SPSS output showing the Shapiro-Wilk test of normality which indicates that prior to log transformation the distribution of CK data (CK1, CK2, CK3) deviated significantly from normal ($P < 0.05$). Following log transformation, all CK data (lnck1, lnck2, lnck3, lnck4) were normally distributed ($P > 0.05$).

Tests of Normality

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
CK1	.281	9	.039	.793	9	.017
CK2	.272	9	.054	.814	9	.029
CK3	.230	9	.188	.770	9	.009
CK4	.211	9	.200*	.893	9	.213
Inck1	.200	9	.200*	.967	9	.866
Inck2	.186	9	.200*	.895	9	.226
Inck3	.114	9	.200*	.971	9	.902
Inck4	.180	9	.200*	.939	9	.573

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Homogeneity of variance

In each of the four studies included in this thesis, participants were measured prior to (pre) and at intervals after (post) completing a bout of eccentric, muscle damaging exercise. Thus a repeated measures analysis of variance (ANOVA) design was used to analyse the pre and post data. During analysis, the assumption of sphericity was tested using Mauchly's test which tested the hypothesis that the variances of the differences between conditions were equal. If the assumption of sphericity was violated, the Greenhouse Geisser correction factor was applied to the degrees of freedom in order to produce a valid *F*-ratio. Below is an example of an SPSS output showing Mauchly's test of sphericity which indicates that the CK data has violated the assumption of sphericity. This data would be reported using the Greenhouse Geisser corrections to the degrees of freedom as follows: $F_{(GG\ 1.45, 11.63)} = 15.58, P = .001$

Mauchly's Test of Sphericity^b

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon ^a		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
factor1	.158	12.414	5	.031	.485	.564	.333

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

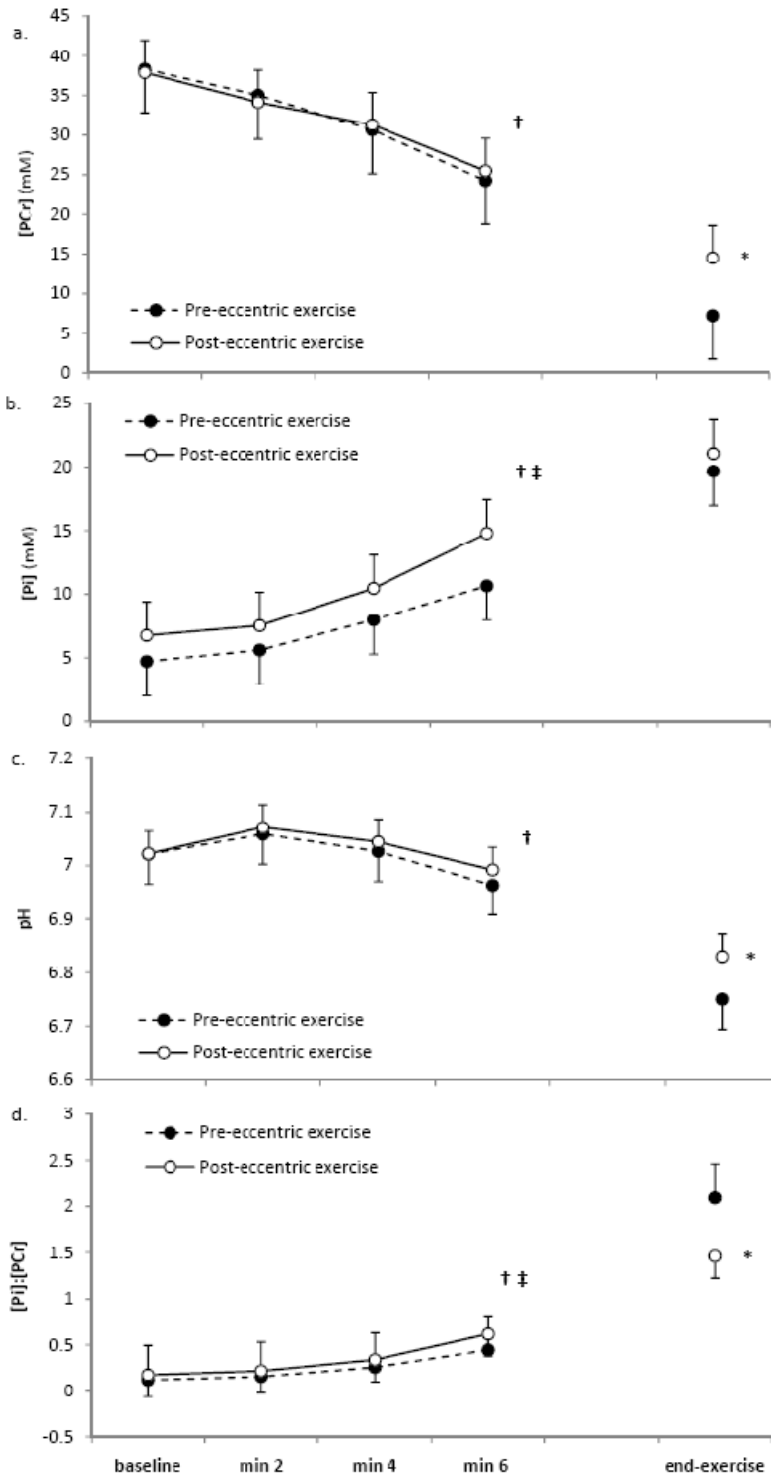
a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
factor1	Sphericity Assumed	9.949	3	3.316	15.583	.000
	Greenhouse-Geisser	9.949	1.454	6.844	15.583	.001
	Huynh-Feldt	9.949	1.693	5.877	15.583	.000
	Lower-bound	9.949	1.000	9.949	15.583	.004
Error(factor1)	Sphericity Assumed	5.108	24	.213		
	Greenhouse-Geisser	5.108	11.629	.439		
	Huynh-Feldt	5.108	13.544	.377		
	Lower-bound	5.108	8.000	.638		

APPENDIX I:



Study 3.

³¹P-MRS muscle metabolic responses

Changes in a. phosphocreatine ([PCr]), b. inorganic phosphate ([Pi]), c. pH and d. [PCr]:[Pi] during incremental exercise, pre- and post-eccentric, muscle damaging exercise.

Values presented are mean (\pm SEM). End exercise [Pi]:[PCr] ratio data were not normally distributed therefore values are presented as medians.

† Significant main effect for time (resting, 2, 4 and 6 min) for all metabolic responses ($P < 0.05$)

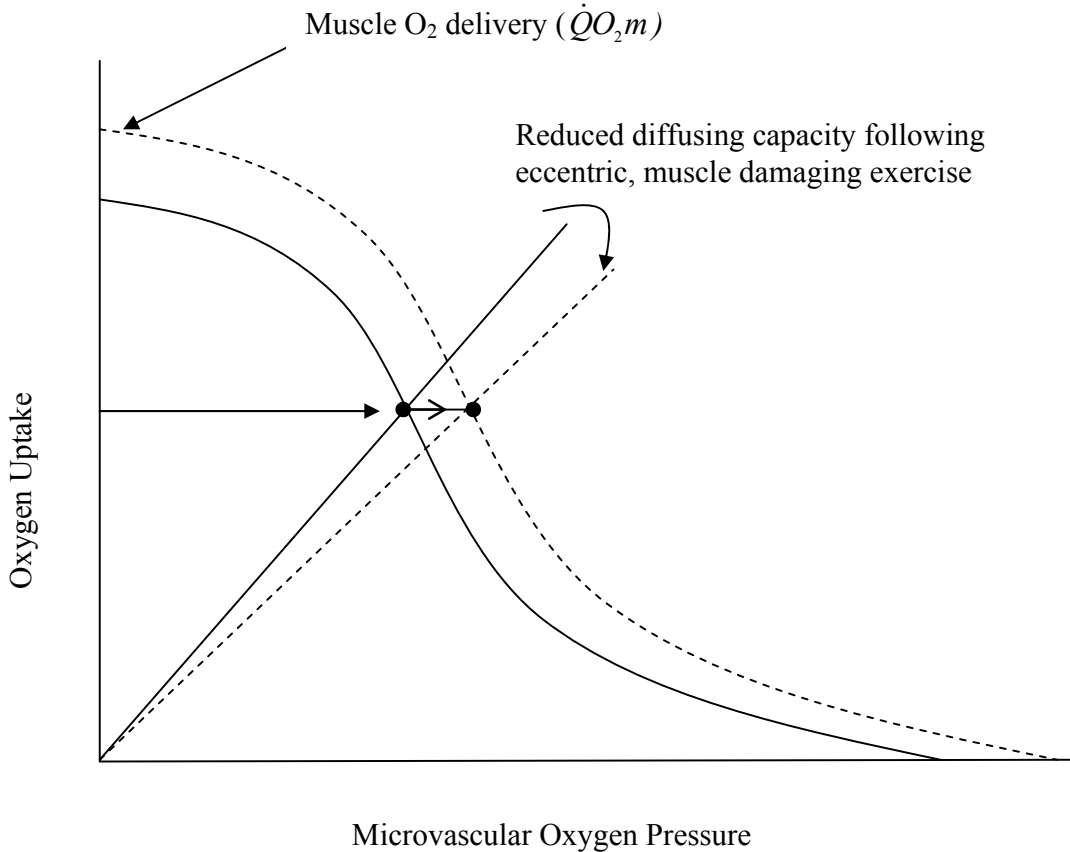
‡ Significant main effect for condition (Pre and Post) for [Pi] and [Pi]:[PCr] ($P < 0.05$)

* End exercise values for [PCr], pH and [Pi]:[PCr] significantly different from Pre-eccentric exercise ($P < 0.05$)

Time to exhaustion (end-exercise) was significantly reduced following muscle damage (519 ± 56 and 459 ± 63 s, pre and post muscle damage respectively, $P < 0.05$).

APPENDIX J:

Schematic demonstrating the reduced diffusing capacity of muscle following eccentric muscle damaging exercise (modelled after Wagner et al., 1996).



The curved lines denote mass balance according to the Fick principle:

$$(\dot{V}O_{2m} = \dot{Q}m (CO_{2a} - CO_{2mv}))$$

Where $\dot{V}O_{2m}$ is muscle O_2 uptake, $\dot{Q}m$ is muscle blood flow, CO_{2a} is arterial O_2 concentration and CO_{2mv} is microvascular O_2 concentration.

The straight lines projecting from the point of origin denote the diffusing capacity according to Fick's Law of diffusion:

$$(\dot{V}O_{2m} = DO_{2m} (PO_{2mv} - PO_{2intra}))$$

Where PO_{2mv} and PO_{2intra} are the microvascular and intramyocyte mean O_2 partial pressures, respectively.

Note that following eccentric muscle damaging exercise (dashed line) the reduced diffusing capacity is compensated for by an elevated microvascular oxygen pressure (achieved by increased local muscle blood flow) to maintain the same level of oxygen uptake.

Wagner, P. D. (1996). Determinants of maximal oxygen transport and utilization. *Annual Review of Physiology*, 58, 21-50.