

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

# **Metabolic Alterations in Skeletal Muscle Following Eccentric Exercise Induced Damage**

**Jonathan D. Hughes MSc**

**November 2011**

**A thesis submitted in partial fulfilment of the  
requirements for the degree of Doctor of Philosophy**

**Massey University | Palmerston North | New Zealand**

# Abstract

---

Eccentric exercise-induced muscle damage (EEIMD) is experienced following unaccustomed eccentric-biased exercise. Gaps in knowledge on aspects of the metabolic response to EEIMD exist, particularly on *in vivo* metabolism. The aim of this thesis is to provide empirical evidence to advance the scientific knowledge and understanding of EEIMD by investigating the metabolic responses following acute and adaptive bouts of eccentric exercise. Eccentric exercise causes changes to the ultrastructure of skeletal muscle and may alter the ability of the muscle to store and utilise intracellular substrates such as glycogen and intramyocellular lipid (IMCL). Using expired respiratory gases collected during one legged cycling to estimate whole body substrate utilisation, the first study showed that acute bouts of eccentric exercise alter the pattern of substrate selection. The effect of EEIMD on substrate utilisation during one legged cycling revealed significantly higher rates of CHO oxidation in EEIMD and that the CHO oxidation further increased during one legged cycling at 48 hours. This is suggestive of greater reliance upon muscle glycogen during subsequent bouts of exercise. The utilisation of nuclear magnetic resonance (NMR) spectroscopy to measure phosphate compounds and IMCL content of the *vastus lateralis* allowed for examination of changes in substrate storage following exposure to an acute bout of eccentric exercise. The second study showed that, following EEIMD, using proton spectroscopy (<sup>1</sup>H-MRS), alterations occur in the IMCL pool within skeletal muscle with a higher concentration evident in the eccentric leg at 24 hours but the trend had been reversed at 48 hours with higher concentrations of IMCL in the concentric leg at 48 hours. Using phosphorous spectroscopy (<sup>31</sup>P-MRS) there was also a significant alteration for resting phosphate stores with increases in inorganic phosphate concentration ([P<sub>i</sub>]) in EEIMD. Eccentric exercise also alters the physiological response to normal levels of insulin and can be defined as ‘transient insulin resistance’. Repeated eccentric exercise training initiates a protective adaptation so that recovery results in reduced symptoms of damage in the repeat bout compared to the initial bout. The third study investigated; via a standard 75g oral glucose tolerance test (OGTT), whether disruptions to glucose and insulin responses following eccentric exercise could be attenuated after a repeated bout of eccentric exercise. There was no change in the insulin response, in comparison to a control trial, 48 hours after a bout of 100 squats of 30% body mass; this formed the eccentric exercise for the study. A subsequent bout of

the same eccentric exercise did not attenuate the insulin response. It is not known if repeated exposure to eccentric exercise can attenuate increases in indirect measures of intracellular metabolism ( $P_i / PCr$ ) following EEIMD, as seen in study two. Study four utilised  $^{31}P$ -MRS to examine the effect of EEIMD on intramyocellular phosphate stores in skeletal muscle, which had been concentrically or eccentrically trained. The data revealed that increases in skeletal muscle phosphate metabolism were not attenuated following exposure to repeated bouts of eccentric exercise and decrements in force generating capacity of muscle following EEIMD must be mediated by central factors. The four studies have provided novel insights into the influence of eccentric, muscle-damaging exercise on the metabolic response of skeletal muscle.

**Summary Table**

	Study 1 (Chapter 3)	Study 2 (Chapter 4)	Study 3 (Chapter 5)	Study 4 (Chapter 6)
Participants	8 males	6 males	8 males	5 males 1 female
Measure	Whole body substrate oxidation	IMCL, $P_i$ , $P_i / PCr$	Glycaemic response	$P_i$ , $P_i / PCr$
Measurement Tools	Indirect calorimetry	$^1H$ -MRS, $^{31}P$ -MRS	75g 2 hour OGTT	$^{31}P$ -MRS
Eccentric Exercise	Bench Stepping	Isokinetic dynamometry / Quads	Squat exercise	Isokinetic dynamometry / Quads
1 <sup>st</sup> Outcome measure	Increased RER (Reliance on glycogen)	Higher IMCL in EEIMD at 24 hrs.	No change in whole body insulin or glucose response	Increased $P_i$ and $P_i / PCr$ in both conditions
2 <sup>nd</sup> Outcome measure	Muscle performance decrease in EEIMD	Increased $P_i$ and $P_i / PCr$ in EEIMD	CK increase but attenuated in second bout	Attenuated muscle performance and volume

# Acknowledgements

---

To my supervisors Associate Professor Stephen Stannard, Dr Nathan Johnson and Dr Stephen Brown, thank you for your wisdom, time and patience throughout this thesis. Thank you for your guidance and education in the art of scientific writing, study design and ensuring quality, consistency and accuracy in all facets of my work. Thank you for challenging me to develop my knowledge and skills. Finally, I thank you for believing in me.

I need to thank all the participants for their effort and willingness to endure the soreness that accompanied the protocols. Great effort guys!

I would also like to thank the additional people both here at Massey University and at The University of Sydney who have helped with the technical support to guide me through my doctoral journey. Special thanks to Toos Sachinwalla and Dave Walton at Rayscan imaging for the time and effort in collect the MRS data. Dave your knowledge of the workings of a magnet is only a fraction of your knowledge of classic Simpson's episodes. To Ray Patton for keeping the dynamometer functioning when it seemed to be exploding under the stress of the eccentric exercise.

Finally, to my wife Helen, it has definitely been a journey and one I couldn't have completed without you. I thank you for your amazing support, help, encouragement and above all the time that you have dedicated to me and allowed me to dedicate to the early mornings and trips to Sydney to collect data. To Cameron and Carys, even though you weren't with me at the start of this journey your arrival has allowed me some wonderful moments of respite from the task of writing. I thank you all for reminding me of what is important in life.

# List of publications and presentations

---

## Peer Reviewed Journals

**Hughes, J. D.**, Johnson, N. A., Brown, S. J., Sachinwalla, T., Walton, D. W. and Stannard, S. R. (2010). Effects of eccentric exercise-induced muscle damage on intramyocellular lipid concentration and high energy phosphates. *European Journal of Applied Physiology*, 110(6), 1135-1141.

## Conference Papers

**Hughes, J. D.**, Johnson, N. A., Brown, S. J., Chapman, P. G. and Stannard, S. R. (2009). Substrate utilisation in eccentric exercise induced damaged muscle. *Proceedings of the New Zealand Sports Medicine & Science Conference*, pp. 74.

**Hughes, J. D.**, Johnson, N. A., Brown, S. J. and Stannard, S. R. (2008). Eccentric exercise induced muscle damage and intramyocellular lipid. *Proceedings of the Medical Sciences Congress*, pp. 64-64.

**Hughes, J. D.**, Johnson, N. A. and Stannard, S. R. (2008). Eccentric exercise induced muscle damage and Intramyocellular lipid concentration. *Post Graduate Sport Research Conference*, pp. 30-32.

# Contents

---

Abstract .....	i
Acknowledgements .....	iv
List of publications and presentations .....	vi
Contents .....	viii
List of abbreviations .....	xii
List of tables .....	xvii
List of figures .....	xix
1. Introduction .....	1
2. Literature review .....	7
2.1 Markers of change in response to acute bouts of eccentric exercise .....	8
2.1.1 Direct evidence of EEIMD – histological evidence .....	8
2.1.2 Indirect evidence of EEIMD .....	10
2.1.2.1 Muscle enzyme and protein leakage .....	10
2.1.2.2 Calcium homeostasis .....	11
2.1.2.3 Inflammatory response .....	13
2.1.2.4 Delayed onset of muscle soreness .....	13
2.1.3 Impaired metabolism .....	15
2.1.3.1 Glucose and insulin .....	15
2.1.3.2 Glycogen and exercise .....	19
2.1.3.3 Intramyocellular lipid .....	21
2.1.3.4 High energy phosphates .....	26
2.2 Magnetic resonance spectroscopy .....	29
2.2.1 Intramyocellular lipid .....	30
2.2.2 High energy phosphates .....	32
2.2.3 Quantifying metabolites using <sup>31</sup> P-MRS .....	34
2.2.4 Quantifying the metabolic consequences of EEIMD with <sup>31</sup> P-MRS .....	36

2.3	Adaptations in response to repeated bouts of eccentric exercise .....	38
2.3.1	Evidence of adaptations .....	38
2.3.2	Theories on mechanisms for adaptive process.....	39
2.4	Aims and objectives .....	41
3.	Indirect measures of substrate utilisation following eccentric exercise induced muscle damage .....	43
3.1	Abstract .....	44
3.2	Introduction .....	45
3.3	Methodology .....	46
3.4	Results .....	52
3.5	Discussion .....	55
4.	Effects of eccentric exercise induced muscle damage on intramyocellular lipid concentration and high energy phosphates .....	58
4.1	Abstract .....	59
4.2	Introduction .....	60
4.3	Methodology .....	62
4.4	Results .....	69
4.5	Discussion .....	72
5.	Repeat bout effect on eccentric exercise induced muscle damage and glycaemic response.....	75
5.1	Abstract .....	76
5.2	Introduction .....	77
5.3	Methodology .....	79
5.4	Results .....	84
5.5	Discussion .....	87

6.	Effect of eccentric versus concentric training on metabolic measures following eccentric exercise induced muscle damage.....	91
6.1	Abstract .....	92
6.2	Introduction .....	93
6.3	Methodology .....	95
6.4	Results .....	99
6.5	Discussion .....	102
7.	Discussion.....	106
7.1	Whole body substrate utilisation .....	107
7.2	Whole body substrate storage.....	109
7.3	Insulin response and adaptation following EEIMD .....	110
7.4	High energy phosphates .....	113
7.5	Conclusion.....	115
8.	References.....	118
9.	Appendices.....	158
9.1	Study One .....	159
9.2	Study Two .....	166
9.3	Study Three .....	171
9.4	Study Four .....	179
9.5	Publication.....	184
9.6	Statement of Contribution .....	185

## List of abbreviations

---

<b>ADP</b>	Adenosine diphosphate
<b>AMARES</b>	Advanced method for accurate, robust and efficient spectral fitting
<b>AMP</b>	Adenosine monophosphate
<b>AMPK</b>	AMP-activated protein kinase
<b>ANOVA</b>	Analysis of variance
<b>ATP</b>	Adenosine triphosphate
<b>AUC</b>	Area under the curve
<b>B<sub>0</sub></b>	Magnetic field
<b>bFGF</b>	Basic fibroblast growth factor
<b>BSE</b>	Bench stepping exercise
<b>C</b>	Carbon
<b>Ca<sup>2+</sup></b>	Calcium
<b>CCB</b>	Calcium channel blockers
<b>CHO</b>	Carbohydrate
<b>CK</b>	Creatine kinase
<b>CMJ</b>	Countermovement jump
<b>CO<sub>2</sub></b>	Carbon dioxide
<b>COM</b>	Centre of motion
<b>Con</b>	Concentric
<b>ConTr</b>	Concentric training
<b>Cr</b>	Creatine
<b>CSA</b>	Cross sectional area
<b>CV</b>	Coefficient of variance
<b>DOMS</b>	Delayed onset muscle soreness

<b>E-C</b>	Excitation-contraction coupling
<b>Ecc</b>	Eccentric
<b>EccTr</b>	Eccentric training
<b>EE</b>	Eccentric exercise
<b>EEIMD</b>	Eccentric exercise induced muscle damage
<b>EMCL</b>	Extramyocellular lipid
<b>FADH<sub>2</sub></b>	Flavin adenine dinucleotide
<b>FFAs</b>	Free fatty acids
<b>FID</b>	Frequency induction decay
<b>GLUT-1</b>	Glucose transporter type 1
<b>GLUT-4</b>	Glucose transporter type 4
<b>H</b>	Hydrogen
<b>HL</b>	Hydroxylysine
<b><sup>1</sup>H-MRS</b>	Proton magnetic resonance spectroscopy
<b>HP</b>	Hydroxyproline
<b>HR</b>	Heart rate
<b>HSD</b>	Honestly significant difference
<b>HSL</b>	Hormone sensitive lipase
<b>IMCL</b>	Intramyocellular lipid
<b>IMTG</b>	Intramuscular triacylglycerols
<b>IRS-1</b>	Insulin receptor substrates 1

<b>IRS-2</b>	Insulin receptor substrates 2
<b>LDH</b>	Lactate dehydrogenase
<b>LED</b>	Light emitting diode
<b>LPL</b>	Lipoprotein lipase
<b>MG<sup>+2</sup></b>	Magnesium
<b>MRI</b>	Magnetic resonance imaging
<b>MRS</b>	Magnetic resonance spectroscopy
<b>MVC</b>	Maximal voluntary contraction
<b>NADH</b>	Nicotinamide adenine dinucleotide
<b>NMR</b>	Nuclear magnetic resonance
<b>NOE</b>	Nuclear overhauser enhancement
<b>O<sub>2</sub></b>	Oxygen
<b>OGTT</b>	Oral glucose tolerance test
<b><sup>31</sup>P-MRS</b>	Phosphorus magnetic resonance spectroscopy
<b>P</b>	Phosphorus
<b>PASW</b>	Predictive analytics software
<b>PCr</b>	Phosphocreatine
<b>PDE</b>	Phosphodiesterases
<b>P<sub>i</sub></b>	Inorganic phosphate
<b>PI3-kinase</b>	Phosphatidylinositol 3-kinase
<b>P<sub>i</sub> / PCr</b>	Inorganic phosphate / phosphocreatine ratio
<b>PME</b>	Phosphomonesters

<b>PRESS</b>	Point resolved spectroscopy
<b>QUEST</b>	Quantitation based on quantum estimation
<b>RBE</b>	Repeat bout effect
<b>RER</b>	Respiratory exchange ratio
<b>ROM</b>	Range of motion
<b>RPE</b>	Ratings of perceived exertion
<b>SD</b>	Standard deviation
<b>SR</b>	Sarcoplasmic reticulum
<b>T1</b>	Longitudinal relaxation time
<b>T2</b>	Transverse relaxation time
<b>TCr</b>	Total creatine
<b>TCr<sub>BASAL</sub></b>	Total basal muscle creatine
<b>TE</b>	Echo time
<b>TG</b>	Triacylglycerol
<b>TNF<math>\alpha</math></b>	Tumour necrosis factor- $\alpha$
<b>TR</b>	Repetition time
<b>VAS</b>	Visual analogue scale
<b><math>\dot{V} O_{2max}</math></b>	Maximal oxygen consumption
<b>WL1</b>	Work load 1
<b>WL2</b>	Work load 2

## List of tables

---

## **Chapter 2**

Table 2.1 Representative values of resting vastus lateralis intramyocellular lipid (IMCL) content taken from mixed muscle fibres in active and trained populations.

Table 2.2 Representative values of resting vastus lateralis PCr content taken from mixed muscle fibres via needle biopsy technique in active populations.

## **Chapter 3**

Table 3.1 Measures of knee extensor muscular strength and perceived muscle soreness following strenuous eccentric exercise.

## **Chapter 4**

Table 4.1 *Quadriceps* resting muscle volumes and metabolite concentrations in *V. lateralis* of control and damaged legs.

## **Chapter 5**

Table 5.1 Measures of knee extensor muscular strength, countermovement jump performance and perceived muscle soreness.

## **Chapter 6**

Table 6.1 Maximal voluntary contractions for ConTr and EccTr legs.

Table 6.2 *Quadriceps* muscle volumes and resting metabolite concentrations in ConTr and EccTr legs.

## List of figures

---

## **Chapter 1**

Figure 1.1 The force-velocity relationship of skeletal muscle.

Figure 1.2 Postulated sequence of events leading to eccentric exercise induced muscle damage (EEIMD).

Figure 1.3 The relationship between length and tension in skeletal muscle.

Figure 1.4 Critical stages in the increase of myofilament overlap.

## **Chapter 2**

Figure 2.1 Electron micrographs of longitudinal sections illustrating muscle damage following eccentric exercise in human skeletal muscle.

Figure 2.2 Insulin and contraction translocation of GLUT-4.

Figure 2.3 Quantification of IMCL by MRS.

Figure 2.4 A normal resting phosphorus spectrum of muscle.

## **Chapter 3**

Figure 3.1 Schematic presentation of the experimental protocol.

Figure 3.2 Schematic presentation of the one legged cycling protocol.

Figure 3.3 CK activity following Bench Stepping Exercise (BSE).

Figure 3.4 Respiratory exchange ratio (A), CHO oxidation (B) and fat oxidation (C) in Con and Ecc legs following BSE.

## **Chapter 4**

- Figure 4.1 Schematic presentation of the experimental protocol.
- Figure 4.2 Positioning of the P-10 receiver coil and leg positioning for MRS measurements.
- Figure 4.3 Representative mid-quadriceps axial plane MRI from 1 subject after eccentric exercise bout.
- Figure 4.4 IMCL: Cr in both experimental (eccentric) and control (concentric) legs at 24 and 48 hours post exercise.
- Figure 4.5 Representative  $^1\text{H}$ -MRS spectra for both conditions (Ecc and Con) at both time points (24 and 48 hours) of human skeletal muscle.

## **Chapter 5**

- Figure 5.1 Schematic presentation of the experimental protocol.
- Figure 5.2 Percentage change in Serum CK activity in response to the initial squat exercise (SE1) and the subsequent bout (SE2).
- Figure 5.3 (A) Glucose and (B) Insulin response to an oral glucose-tolerance test (OGTT).

## **Chapter 6**

- Figure 6.1 Schematic presentation of the experimental protocol.