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Metabolic Alterations in Skeletal Muscle Following Eccentric Exercise Induced Damage

Jonathan D. Hughes MSc

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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 ($^1$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 ($^{31}$P-MRS) there was also a significant alteration for resting phosphate stores with increases in inorganic phosphate concentration ($[P_i]$) 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.

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

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List of abbreviations
ADP  Adenosine diphosphate
AMARES  Advanced method for accurate, robust and efficient spectral fitting
AMP  Adenosine monophosphate
AMPK  AMP-activated protein kinase
ANOVA  Analysis of variance
ATP  Adenosine triphosphate
AUC  Area under the curve

$B_0$  Magnetic field
bFGF  Basic fibroblast growth factor
BSE  Bench stepping exercise

C  Carbon
$Ca^{2+}$  Calcium
CCB  Calcium channel blockers
CHO  Carbohydrate
CK  Creatine kinase
CMJ  Countermovement jump
CO$_2$  Carbon dioxide
COM  Centre of motion
Con  Concentric
ConTr  Concentric training
Cr  Creatine
CSA  Cross sectional area
CV  Coefficient of variance

DOMS  Delayed onset muscle soreness
E-C  Excitation-contraction coupling
Ecc  Eccentric
EccTr Eccentric training
EE   Eccentric exercise
EEIMD Eccentric exercise induced muscle damage
EMCL Extramyocellular lipid

FADH$_2$  Flavin adenine dinucleotide
FFAs  Free fatty acids
FID  Frequency induction decay

GLUT-1  Glucose transporter type 1
GLUT-4  Glucose transporter type 4

H  Hydrogen
HL  Hydroxylysine
$^1$H-MRS  Proton magnetic resonance spectroscopy
HP  Hydroxyproline
HR  Heart rate
HSD  Honestly significant difference
HSL  Hormone sensitive lipase

IMCL  Intramyocellular lipid
IMTG  Intramuscular triacylglycerols
IRS-1  Insulin receptor substrates 1
IRS-2 Insulin receptor substrates 2

LDH Lactate dehydrogenase

LED Light emitting diode

LPL Lipoprotein lipase

MG$^{+2}$ Magnesium

MRI Magnetic resonance imaging

MRS Magnetic resonance spectroscopy

MVC Maximal voluntary contraction

NADH Nicotinamide adenine dinucleotide

NMR Nuclear magnetic resonance

NOE Nuclear overhauser enhancement

O$_2$ Oxygen

OGTT Oral glucose tolerance test

$^{31}$P-MRS Phosphorus magnetic resonance spectroscopy

P Phosphorus

PASW Predictive analytics software

PCr Phosphocreatine

PDE Phosphodiesters

P$_i$ Inorganic phosphate

PI3-kinase Phosphatidylinositol 3-kinase

P$_i$ / PCr Inorganic phosphate / phosphocreatine ratio

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