Metabolic Alterations in Skeletal Muscle Following Eccentric Exercise Induced Damage

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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.

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	^{1}H-MRS, ^{31}P-MRS	75g 2 hour OGTT	^{31}P-MRS
Eccentric Exercise	Bench Stepping	Isokinetic dynamometry / Quads	Squat exercise	Isokinetic dynamometry / Quads
1st 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
2nd 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

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List of publications and presentations

Peer Reviewed Journals

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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.

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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

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

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

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1. Introduction

An eccentric muscle contraction occurs when the muscle is forced to lengthen by an external force greater than the opposing internal force generated by the muscle. Much of the external energy being applied is absorbed by the muscle. This is in contrast to a concentric muscular contraction, which is when the force actively produced by the muscle exceeds the external load, the muscle shortens and external work is done. Eccentric muscle actions are common in sporting activities, providing the halting forces required in weightlifting, racquet sports and in many team games where rapid deceleration and changes in direction are necessary.

A characteristic of an eccentric muscle contraction is that the maximal tension generated exceeds that of a maximal concentric contraction by the same muscle (Figure 1.1) (Bigland-Ritchie and Woods, 1976, Katz, 1939, Komi and Rusko, 1974, Singh and Karpovic, 1966, Stauber, 1989).

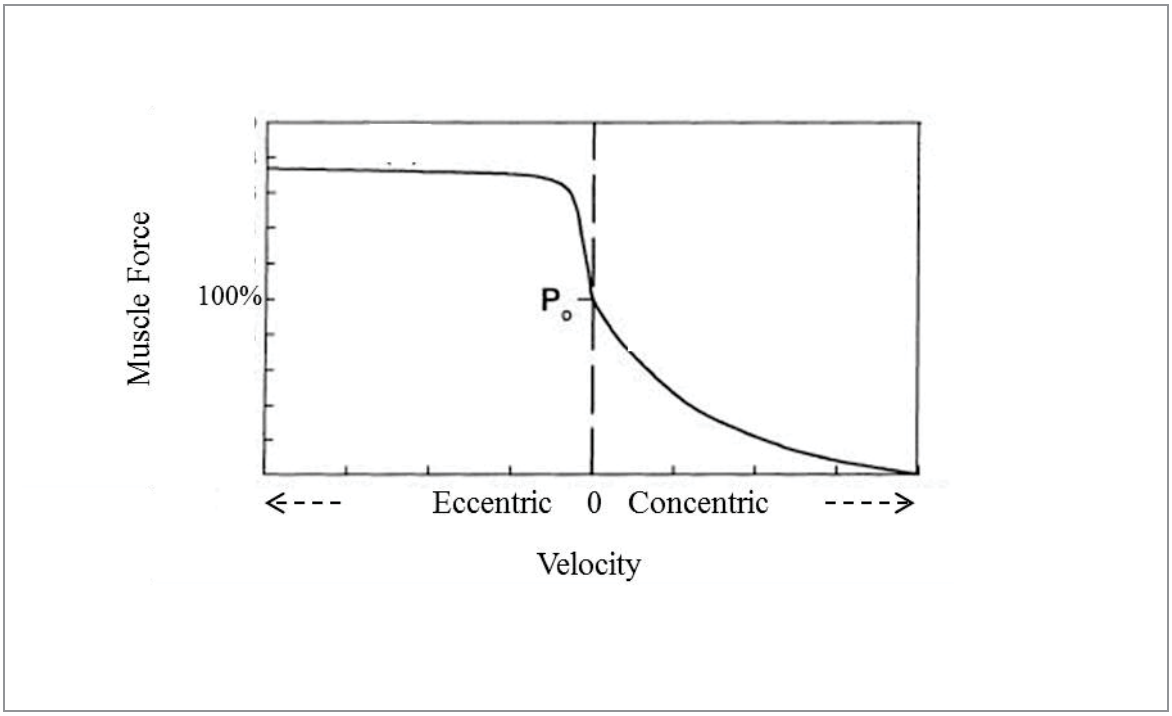

Figure 1.1. The force-velocity relationship of skeletal muscle. P_o represents maximal (100%) isometric force. Adapted from (Allen, 2001), p. 312.

The energy requirement for an eccentric contraction is different to both isometric and concentric contractions of the same tension (Curtin and Davies, 1973, Wilkie, 1968).

The energy cost for mechanical output of a muscle *in situ* has shown the lowest energy cost for mechanical output observed during eccentric contraction, with the highest cost coming from concentric contraction (Beltman et al., 2004b). Measurement of ATP turnover using ^{31}P magnetic resonance spectroscopy (^{31}P MRS) has shown that the mechanical efficiency of eccentric muscular contractions are twice as efficient (35%) as concentric muscular contractions (15%) of an equivalent workload (Ryschon et al., 1997). However, this may be because much of the work performed in eccentric loading is done by the stretching of connective tissue, which has elastic properties. This would not be reflected in increased energy expenditure (i.e. increased metabolic rate) but would appear as heat in the muscle as a result of internal friction. An explanation for this phenomenon is offered through the cross-bridge model (Huxley, 1957), which suggests that during an eccentric contraction there will be cross-bridges that are being stretched and mechanically broken without the need for hydrolysis of adenosine triphosphate (ATP) (Flitney and Hirst, 1978).

An interaction of high force and the length at which force is generated is closely related to the occurrence of damage to the muscle. Long fast, stretching muscle actions cause the greatest magnitude of damage. Lieber and Friden (1993) reported that the magnitude of active strain, rather than high force alone contributed to contractile and morphological changes associated with contraction-induced damage. Sarcomeres within a muscle fibre are variable in length but each have an optimal length for generating maximal tension. The non-uniform length characteristic means that sarcomeres within the same myofibre function on all parts of the length tension curve. This causes some to function on the plateau or descending limb of the curve where their length is beyond optimal for force generation, which means that sarcomere equilibrium is unstable. The heterogeneous distribution of sarcomere lengths may explain the focal nature of damage to muscle. The interaction between the length-tension and force-velocity relationships contributes to mechanical disruption of myofilament, which is an influential determinant of the magnitude of the disruption to the sarcomere.

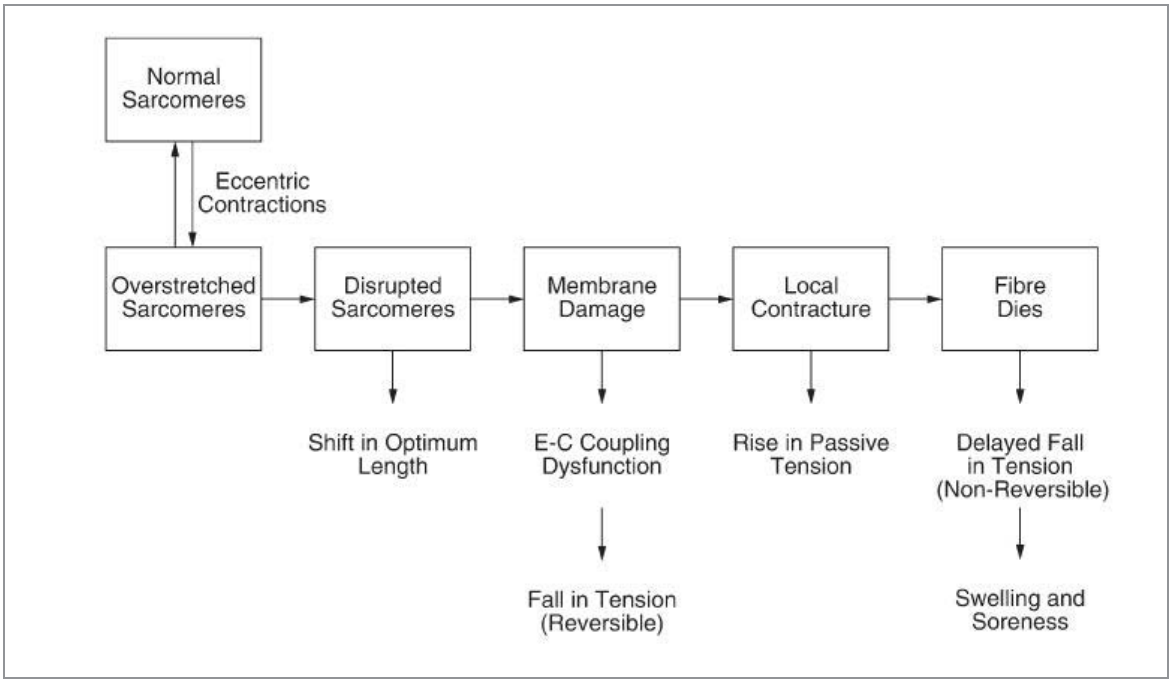

Figure 1.2. Postulated sequence of events leading to eccentric exercise induced muscle damage (EEIMD). From Proske & Morgan (2001), p. 334.

For the purpose of this thesis, 'muscle damage' will include all changes that follow eccentric exercise and will be termed 'eccentric exercise induced muscle damage' (EEIMD). The suggested initial event in EEIMD is an over-extension of sarcomeres beyond their yield point (Figure 1.2), which is termed the 'popping sarcomere hypothesis' (Morgan and Proske, 2004, Morgan, 1990). EEIMD occurs as a result of non-uniform lengthening of sarcomeres when a muscle is stretched beyond an optimum length for force generation, especially on the descending limb of the length-tension curve (Figures 1.3 & 1.4). Sarcomeres that extend beyond the point of optimal length become longer, weaker and consequently less able to hold tension. The sarcomeres will then lengthen rapidly until a point of passive tension is reached (Morgan and Proske, 2004, Proske and Morgan, 2001). Failure of the overstretched sarcomeres to hold tension following an eccentric contraction increases the relative tension on the functional sarcomeres. In addition, the process of lengthening, weakening and failure will repeat itself on subsequent weak sarcomeres in the myofibril. This continues for the duration of loading caused by eccentric contractions (Morgan, 1990, Proske et al., 2004). Following an eccentric contraction, the majority of sarcomeres re-interdigitate. However, the damaged sarcomeres fail to re-interdigitate, which leads to myofilament

disruption. Disruption to the sarcomere is observed in the form of z-band streaming, broadening and total disruption (Fridén et al., 1983b). Disruption also leads to changes in the sarcolemma causing changes to the permeability of the cell membrane and basal lamina. Less recruitment of motor units takes place during eccentric contractions than during concentric contractions (Beltman et al., 2004a). This can be interpreted as increased force per cross-bridge, predisposing the contractile proteins to failure. Repeated eccentric contractions then place sufficient mechanical stress on the muscle to induce failure in some sarcomeres.

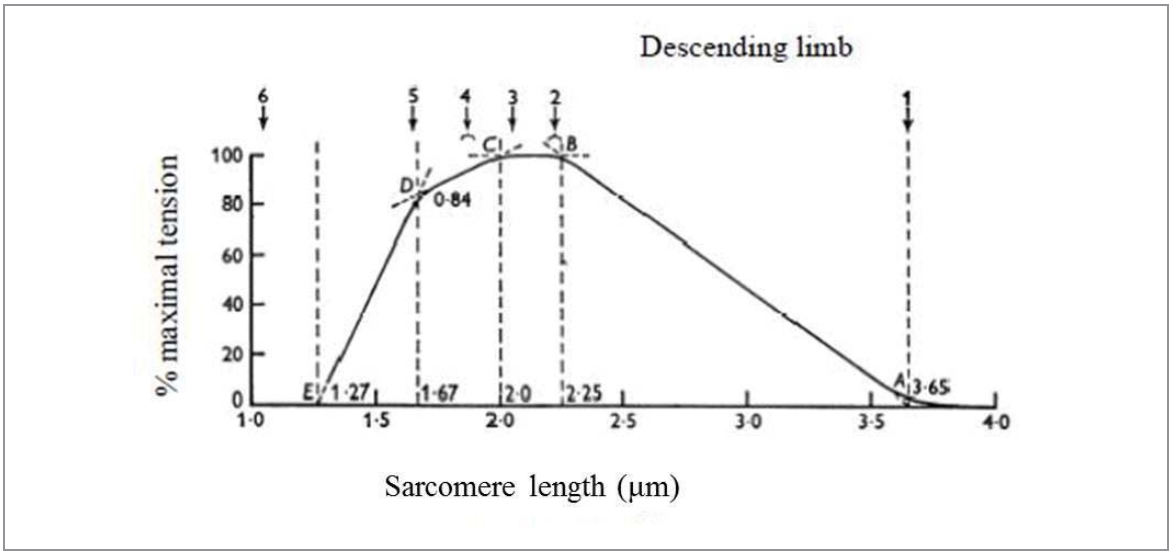

Figure 1.3. The relationship between length and tension in a sarcomere. Adapted from Gordon et al. (1966), p.185.

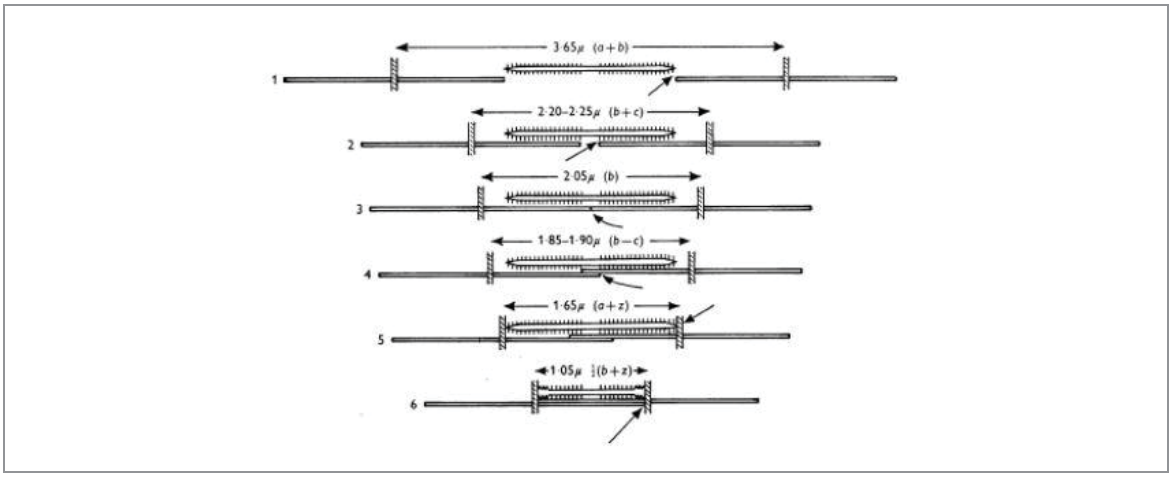

Figure 1.4. Critical stages in the increase of myofilament overlap corresponding to key points (1-6) labelled on the length-tension curve in Figure 1.3. Adapted from Gordon et al. (1966), p.186.

Following eccentric contractions by the muscle, a loss of free calcium (Ca^{2+}) homeostasis and an initiation of Ca^{2+} overload has been shown (Armstrong et al., 1991). Cytosolic Ca^{2+} levels are closely regulated within muscle cells by a number of intracellular Ca^{2+} buffering and translocation mechanisms. When these mechanisms are overwhelmed following muscle damage, several intrinsic degradative pathways are activated. These include the activation of a number of Ca^{2+}-dependent proteolytic and phospholipolytic pathways, which degrade structural and contractile myofibre proteins as well as the myofibre membrane (Kuipers, 1994). These two initial phases of muscle fibre injury are followed by a phagocytic phase, during which the inflammatory response allows the removal of damaged tissue and the regeneration of damaged muscle fibres to occur (Koh et al., 2003, Stupka et al., 2001).

The physiological and functional responses to EEIMD have become well characterised through extensive research. Less is known about the metabolic response to EEIMD, with much of the research providing equivocal results. The intention of this thesis is to provide empirical evidence to advance the scientific knowledge and understanding of the phenomenon of EEIMD, principally by investigating the metabolic responses to the performance of subsequent bouts of exercise exhibiting EEIMD.

The following review critiques contemporary literature in order to identify reliable knowledge, on which to propose hypotheses related to the metabolic perturbations that arise as a result of EEIMD. Following the review of literature the thesis is comprised of four chapters that contain the experimental studies conducted. Studies 1 and 3 used a total sample of 8 participants. Similarly, studies 2 and 4 used a total sample of 6 participants, 5 of which were common to both studies. Following the experimental chapters there is a general discussion which ties together the findings from the individual experimental chapters.

2. Literature review

2.1 Markers of change in response to acute bouts of eccentric exercise

In EEIMD there are changes in a muscle's ability to perform a number of functions. Immediate changes include reductions in force generating capacity. Delayed changes become evident a number of days later, including increases in plasma levels of muscle proteins and enzymes, swelling, stiffness, inflammation and soreness. When quantifying skeletal muscle damage, it is possible to utilise both direct and indirect markers to assess the extent of damage to the skeletal muscle.

2.1.1 Direct evidence of EEIMD – histological evidence

The needle biopsy technique has provided the opportunity to investigate human skeletal muscle metabolism, particularly changes in substrate concentration that occur due to exercise and nutritional interventions (Bergström, 1962, Hultman et al., 1967). The technique has altered little in the period since being reintroduced by Bergström (1962). Skeletal muscle biopsy samples extracted by needle biopsy are commonly taken from the *vastus lateralis* due to the interior location of major blood vessels and nerves, along with the fact that it is a major locomotor muscle.

Human muscle biopsy data has provided direct evidence of considerable disruption in the ultrastructure of skeletal muscle following unaccustomed eccentric exercise (Fridén et al., 1983b, Fridén et al., 1981, Gibala et al., 1995, Jones et al., 1986, Newham et al., 1983b). Disturbances, which originate in the myofibrillar z-line, are seen as streaming, broadening or total disruption (Figure 2.1). Z-line disruption is the most frequently reported ultrastructural abnormality and may represent the 'weak link' in the myofibril contractile mechanism (Newham et al., 1983b). Reports of greater disruption of type II fibres suggest that these fibres are preferentially damaged during eccentric exercise (Fridén et al., 1983b, Jones et al., 1986, Lieber et al., 1991). Eccentric contractions generate higher levels of mechanical stress than concentric or isometric contractions due to reduced motor unit activation (Enoka, 1996, 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, Nardone et al., 1989). Excessive stress on a smaller number of active fibres leads to them becoming damaged (McHugh et al., 2000).

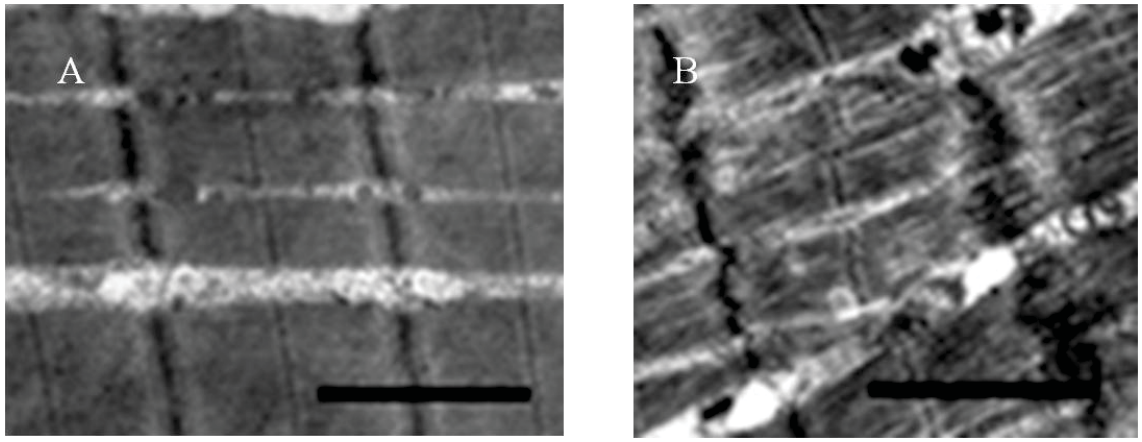

Figure 2.1. Electron micrographs of longitudinal sections of contracted sarcomeres illustrating muscle damage following eccentric exercise in human skeletal muscle. Normal ultrastructural profiles are shown in control muscle (A). Extensive disorganization of the myofibrillar ultrastructure 1 day post eccentric exercise (B). Severe Z-disk damage was associated with A-band disruption and misalignment of myofibrils. (Scale bars: 1 μm). Adapted from Feason et al. (2002), p.301.

There is evidence to suggest that the biopsy procedure itself may produce some changes mistakenly attributed to EEIMD (Malm et al., 2000, Roth et al., 2000) so using this technique may be problematic when investigating skeletal muscle damage. For example, the process of biopsy produces elevated blood creatine kinase (CK) levels, thus potentially confounding the results of CK as a marker of damage (Hikida et al., 1991). Further, it has been reported that injury to the *vastus lateralis* muscle had not repaired following 2 weeks of recovery after the biopsy, thereby the validity and reliability of longitudinal studies can be compromised (Staron et al., 1992). It has also been evidenced that serum CK is not influenced by muscle biopsy in baseline measures (Child et al., 1999), indicating that the biopsy procedure does not produce significant muscle damage. The limitation of investigation of structural markers of EEIMD is the

necessity to undertake biopsy procedures, which are invasive and given that distribution of damaged tissue can be variable within the muscle can be a further limitation. Consequently, the utilisation of indirect markers of muscle damage, such as circulating biochemical and metabolic markers, which can be investigated without trauma to the muscle, have become predominant in the literature.

2.1.2 Indirect evidence of EEIMD

Indirect markers are less invasive than direct muscle sampling through biopsy and allow for repeated measurement over many days and can provide insight regarding the muscles performance following damaging exercise.

2.1.2.1 Muscle enzyme and protein leakage

Compromised sarcolemmal integrity, as a result of EEIMD, produces an efflux of muscle proteins and enzymes into the bloodstream (Hortobágyi and Denahan, 1989). Serum activity of muscle enzymes or proteins can be used as markers of the functional status of muscle tissue. CK, lactate dehydrogenase (LDH), aldonase, myoglobin, troponin, aspartate aminotransferase and carbonic anhydrase (CAIII) have been indicated as serum markers of muscle damage.

An increased activity of CK is assumed to indicate sarcolemma disruption (Hortobágyi and Denahan, 1989). Because CK is a very large molecule (80000 Da), CK should not be able to enter the blood directly due to the supposed small pore radius in the capillaries (40-70 Å) but is thought to be released into the blood stream via the lymph system. This explains the delayed CK activity peak following eccentric exercise. However, Paaske and Sejrsen (1989) found a larger pore radius in the capillaries of 145-160 Å rather than 40-70 Å, which would allow CK to enter the blood stream directly from the interstitial space.

CK activity is one of the most widely used indicators of EEIMD. The CK enzyme is composed of three cytoplasmic isoenzymes; CK-MM, found in skeletal muscle; CK-BB found in the brain; and CK-MB found in cardiac muscle (Brancaccio et al., 2007). An increase in circulating CK is widely used as an indicator of cell damage and permeability (Evans et al., 1986). The time course of CK appearance in the blood

following muscle damage appears to be dependent on the damage protocol employed. Following eccentrically biased whole-body exercise, such as downhill running, weightlifting or plyometric exercise, plasma CK levels peak 24 to 48 hours after completion of exercise (Byrne and Eston, 2002, Chen et al., 2007, Eston et al., 1996, Horita et al., 1999, Twist and Eston, 2005). High-force eccentric exercise using isokinetic dynamometers induces a delayed response with serum CK activity peaking 4 to 5 days following exercise (Chen, 2003, Clarkson et al., 1992, Nosaka and Clarkson, 1992, Zainuddin et al., 2005a). Interpretation of the CK response to EEIMD is complicated by high intersubject variability despite similar decrements in contractile force (Clarkson and Ebbeling, 1988, Hortobágyi and Denahan, 1989). Therefore, while it is possible to hypothesise a relationship between the loss of sarcolemmal integrity, an increase in plasma CK activity and loss of muscle function, there is no evidence that plasma CK activity accurately reflect the extent of myofibre damage caused (Nosaka and Clarkson, 1992).

Although CK activity is widely used as an indicator of muscle damage, it is considered a poor indicator of the magnitude of muscle damage, as it often correlates poorly with other markers of EEIMD (Chapman et al., 2006, Clarkson et al., 1986, Fridén and Lieber, 2001, Newham et al., 1983a, Warren et al., 1999). Also, these blood concentrations would reflect not only their release into the circulation but also their removal. Any alterations in blood flow to the tissue that clears this enzyme will affect the blood CK activity. The use of CK is therefore appropriate as one of many biomarkers to determine whether muscle damage has occurred but should be used with caution when determining the severity of muscle damage.

2.1.2.2 *Calcium homeostasis*

Myofibril damage in animal models of EEIMD has been associated with the loss of intracellular Ca^{2+} homeostasis, in part due to disturbances of the muscle membrane (Armstrong, 1990). The excitation-contraction coupling (E-C) process is the sequence of events that starts with the release of acetylcholine at the neuromuscular junction and ends with the release of Ca^{2+} from the sarcoplasmic reticulum (SR) into the sarcoplasm (Edwards et al., 1977). The disruption of the SR (Byrd, 1992, Fridén and Lieber, 1996) increases SR membrane permeability and is responsible for increases in intracellular

Ca^{2+} concentration ($[Ca^{2+}]_i$) (Armstrong, 1984). Increased cytosolic Ca^{2+} may then contribute to the further degradation of the muscle tissue by stimulating the release of Ca^{2+}-activated neutral proteases such as calpain, which have been shown to damage z-line-associated proteins (Belcastro, 1993, Belcastro et al., 1998, Busch et al., 1972).

Investigations employing a human model have produced conflicting results. For example, Nielsen et al. (2005) observed no change in SR function from EEIMD, while others (Enns et al., 1999) reported no immediate change in SR Ca^{2+} uptake despite prolonged alterations in SR function in the 2 to 14 day recovery period. The administration of calcium channel blockers (CCB) in animal models of EEIMD has been reported to reduce or prevent the rise in intracellular Ca^{2+} and subsequent injury (Armstrong et al., 1993, Duan et al., 1990, Duarte et al., 1992, Soza et al., 1986). 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, 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).

Investigation of EEIMD in mouse soleus muscle (Warren et al., 1993) has provided stronger indications that eccentric contractions cause E-C coupling failure (Warren et al., 1993). These authors performed *in vitro* eccentric contractions on the muscles, which were then exposed to a caffeine probe. Caffeine acts to increase free cytosolic $[Ca^{2+}]_i$ by instigating Ca^{2+} release from the SR. The eccentric contractile activity caused a decline in the tetanic force while the force produced by the caffeine probe was unaltered by eccentric exercise. This indicated that it was a failure in the Ca^{2+} release rather than mechanic factors that caused the force reduction. These findings have been supported in further research (Balnave and Allen, 1995), which found that through moderate eccentric protocols, tetanic force could be restored with caffeine. This again suggested that structural damage was minimally affecting muscle performance. However, when the muscle was stretched further and for a greater number of contractions, there was stronger evidence for structural damage (force that could not be improved with caffeine) and a reduction in Ca^{2+} release.

2.1.2.3 Inflammatory response

Following EEIMD, inflammatory cells such as neutrophils and macrophages infiltrate the muscle in order to remove necrotic tissue and initiate the process of muscle repair and regeneration (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., 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 are able to permeate human skeletal muscle within an hour following eccentric exercise and remain present for up to 5 days (Fielding et al., 1993). However, it is more common that significant increases last up to 24 hours (Beaton et al., 2002, MacIntyre et al., 1996, MacIntyre et al., 2001). The accumulation of neutrophils may activate macrophages resident in the muscle and attract further macrophage invasion. Whilst macrophages are phagocytic, they may also promote repair and regeneration via the release of cytokines known to cause myoblast proliferation *in vitro* (Hawke and 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 muscle damaged from eccentric biased exercise (Schneider and Tiidus, 2007).

Investigation into the inflammatory response to EEIMD in humans and animals remains controversial 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 (Peake et al., 2005, Schneider and Tiidus, 2007, Tidball, 2005).

2.1.2.4 Delayed onset of muscle soreness

The delayed soreness experienced after damaging exercise is another change that is symptomatic of EEIMD. The characteristics of this soreness include sensations of stiffness and pain that follow eccentric exercise (Hough, 1902), which are not immediately felt post exercise. The intensity and sensation of discomfort increases in the first 24 hours following the cessation of exercise, peaking between 24 and 72 hours and subsiding / disappearing by 5 to 7 days post exercise (Armstrong, 1984, Clarkson

and Tremblay, 1988, Jones et al., 1986). This phenomenon is commonly termed delayed-onset of muscle soreness (DOMS). The pain felt refers to muscular pain when the tissue is palpitated or made to contract and is classified as a type I muscle strain injury. It is characterised by soreness in the muscle 24 to 48 hours after a bout of unaccustomed exercise (Gullick and Kimura, 1996). Although this delayed manifestation of pain is a common symptom of unaccustomed muscular activity the mechanisms of the pain are not well understood.

A number of theories have been proposed to help explain the pain associated with DOMS, which include: (i) muscle damage; (ii) connective tissue damage; and (iii) inflammation. These will be outlined in turn:

It is hypothesised that muscle damage produces DOMS-related pain and is focused on the disruption to the contractile elements of skeletal muscle tissue (for further detail see Chapter 1). The mechanical disruption of structural elements is believed to contribute to the stimulation of pain receptors (nociceptors). Nociceptors located in the connective tissue of skeletal muscle and more particularly in the area of the arterioles, capillaries and the musculotendonous junction become stimulated, which leads to the sensation of pain (Smith, 1991). According to this theory, they are the major factor in the sensation of DOMS.

Damage to the connective tissue of skeletal muscle is hypothesised to include the muscle fascia in the development of DOMS. Measurements of urine excretion hydroxyproline (HP) and hydroxylysine (HL) post exercise (Brown et al., 1997b) support this hypothesis with increases seen 2 days post eccentric exercise. HP and HL imino acids are a component of mature collagen and their presence in urine is the result of collagen degradation, whether from overuse or muscle damage injuries (Stauber, 1989). It has also been suggested that damage and shortening of the connective tissue would increase the mechanical sensitivity of muscle nociceptors and cause the pain (Jones et al., 1987).

The role of inflammation in causing DOMS revolves around the evidence of oedema and inflammatory cell infiltration (Evans et al., 1986, Smith, 1991). Following damaging exercise, the proteolytic enzymes contained in the muscle initiate degradation of protein and lipid structures. This rapid breakdown attracts monocytes and

neutrophils to the site of injury / damage (Peake et al., 2005). The next phase is an influx of protein rich fluid into the muscle due to the increased permeability of small blood vessels following eccentric exercise (Smith, 1991). This is thought to increase the osmotic pressure in the cell and can cause pain when type IV sensory neurons are activated (Fridén et al., 1986). It is thought that neutrophils invade injured muscle tissue and their numbers remain elevated for up to 5 days following a damaging event (Fielding et al., 1993). The presence of neutrophils has been thought to result in a proteolytic process that may actually amplify the process of exercise-induced muscle injury (Lowe et al., 1995).

In terms of explaining the pain associated with DOMS, no single theory offers a fully adequate explanation. The stimuli that inform nociceptors and other receptors to evoke DOMS have not been fully elucidated. The magnitude of DOMS does not always reflect the extent of muscle damage and the time course does not always correlate well the time course changes in other indicators of muscle damage.

2.1.3 Impaired metabolism

For the purpose of investigating the effects of skeletal muscle damage on substrate metabolism, it is useful to describe how skeletal muscle obtains substrate sources for energy production in a healthy non-damaged condition.

2.1.3.1 Glucose and insulin

In healthy non-damaged skeletal muscle, glucose enters the cell from the blood by way of glucose transporter proteins. The major transporters in skeletal muscle cells are glucose transporter type 1 (GLUT-1) and glucose transporter type 4 (GLUT-4). Resting glucose transport is carried out by GLUT-1, a protein that has been identified in highly purified sarcolemmal membranes but not in intracellular membranes (Douen et al., 1990, Goodyear et al., 1991). The GLUT-4 protein is stored in intracellular vesicles. Translocation occurs in response to either insulin stimulation or contraction and the effects of both of these stimuli are additive (Garetto et al., 1984, Nesher et al., 1985, Wallberg-Henriksson et al., 1988). Binding of insulin to membrane bound insulin receptors stimulates the phosphorylation of insulin receptor substrates 1 and 2 (IRS-1

and IRS-2). This leads to the activation of phosphatidylinositol 3-kinase (PI3-kinase), which subsequently induces GLUT-4 translocation (Figure 2.2) (Lund et al., 1995). The enzyme AMP-activated protein kinase (AMPK) plays an important role in the induction of GLUT-4 recruitment to the plasma membrane (Merrill et al., 1997). AMPK activity in muscle has been shown to be rapidly activated by exercise or electrically stimulated muscular contractions (Hutber et al., 1997, Vavvas et al., 1997, Winder and Hardie, 1996). The ability of AMPK to stimulate GLUT-4 translocation to the plasma membrane in skeletal muscle occurs via a mechanism distinct from that stimulated by insulin since together insulin and AMPK effects are additive. This increased glucose uptake will result in an increase in glycolysis and ATP production.

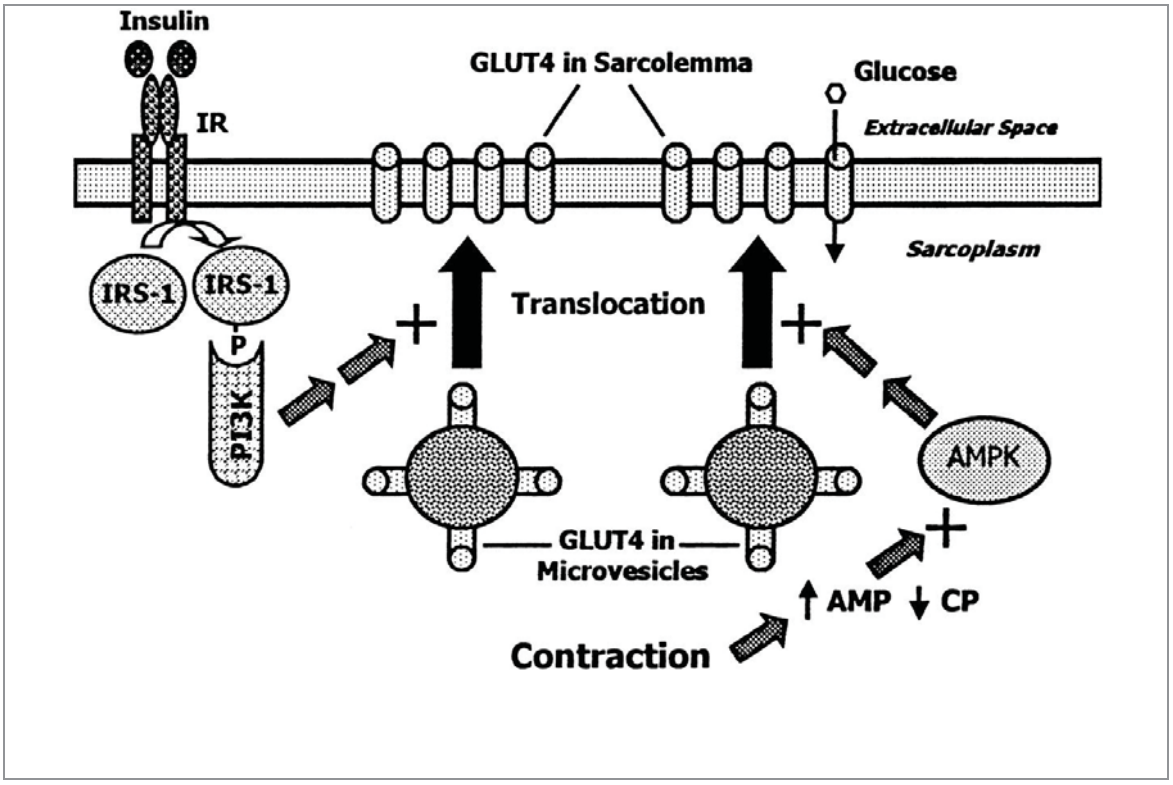

Figure 2.2. Insulin and contraction translocation of GLUT-4. Adapted from (Winder, 2001), p.1022. AMP (Adenosine monophosphate), AMPK (AMP-activated protein kinase), CP (Phosphocreatine), IR (Insulin receptors), IRS-1 (Insulin receptor substrates-1), PI3K (Phosphatidylinositol 3-kinase).

2.1.3.1.1 Response to exercise

Muscular work increases the sensitivity of skeletal muscle to the action of insulin. Studies on healthy males have shown that acute bouts of exercise increase insulin

stimulated whole body glucose uptake, measured via a glucose clamp for at least 16 hours post exercise (Bogardus et al., 1983, Mikines et al., 1988). In addition, acute cycling exercise has been shown to decrease the insulin response to an oral glucose tolerance test (OGTT) (Young et al., 1989), which suggests an increase in peripheral insulin sensitivity.

Chronic contractile activity also appears to potentiate insulin action. Investigation into the effect of an acute bout of exercise on insulin sensitivity for trained and untrained males, 15 hours after cessation of an exercise session (Mikines et al., 1989a) revealed that insulin sensitivity was higher in the trained subjects. Subsequent research looked at the effect of a period of detraining in the insulin sensitivity in trained subjects. Three measures of insulin sensitivity were taken: 15 hours following the last habitual exercise bout; immediately after 60 minutes of exercise at 72 ± 3% $\dot{V}O_{2max}$; and after 5 days of detraining. Insulin sensitivity was found to be similar directly and 15 hours after exercise but decreased to untrained levels after 5 days of detraining (Mikines et al., 1989b). It was found that insulin responsiveness, measured as the maximal insulin-dependent glucose uptake rate during a glucose clamp was the same in all three conditions. This led the authors to conclude that the alteration in maximal insulin action with prolonged endurance training cannot be ascribed to the effect of the last exercise bout.

These findings were extended to a one-legged exercise training model with measurement of glucose clearance of a trained leg (Dela et al., 1992). Participants followed a one-legged training program for 10 weeks on a cycle ergometer. Insulin stimulated glucose uptake was measured in the trained leg 16 hours after the last exercise bout. Subsequently, a single exercise bout was performed with the untrained leg and, once again, insulin stimulated glucose uptake was measured in the trained leg 16 hours later. The findings of the study revealed that the training programme increased glucose clearance of the trained leg at both maximal and sub maximal insulin stimulation, but contrary to the whole body studies no effect was detected of a single bout of one-legged exercise. These findings indicate that skeletal muscle has the ability to enhance its sensitivity to insulin by undertaking training, but that this potential to modify the insulin response is not permanent, given that a short period of de-training can reverse the beneficial effects of exercise.

2.1.3.1.2 Response to eccentric exercise

In contrast to the adaptive response often seen following endurance based predominantly concentric exercise (e.g. cycling), exercise involving a high degree of eccentric activity can be associated with impaired insulin-mediated glucose disposal. This has been demonstrated following a treadmill run to exhaustion (Kirwan et al., 1991) and 20 minutes of downhill running (Kirwan et al., 1992). The latter study observed muscle soreness and a reduction in whole body insulin sensitivity, which was not evident in the control concentric cycling condition. These findings have been corroborated in a further study (del Aguila et al., 2000).

The mechanisms for the decrease in insulin sensitivity associated with EEIMD have focused on the role played by GLUT-4 proteins and the cytokine response to damage. In untrained participants, EEIMD following forced knee flexion led to a 17% decrease in GLUT-4 content of the knee extensor muscle group (Asp et al., 1995). A follow up study by the same group conducted on a group of trained marathon runners found no decrease in GLUT-4 content even though muscle damage was evident in the participants (Asp et al., 1997). The authors attributed this variance in insulin sensitivity to the different methods between the studies that were employed to induce muscle damage. They concluded that the mechanical stress experienced by the untrained participants would be the cause of the decrease in GLUT-4 content. Hence the muscle damage experienced by the trained marathon group was a function of metabolic stress rather than mechanical factors.

The decrease in insulin sensitivity has also been attributed to the inflammatory response related to EEIMD. Particular emphasis has been placed on the cytokine tumour necrosis factor-α (TNFα). A number of studies have shown that levels of a number of cytokines, including TNFα, increase during exercise as an acute response to inflammation (Pedersen and Hoffman-Goetz, 2000, Steinacker et al., 2004, Toft et al., 2002). Using C_2C_{12} muscle cell cultures, insulin stimulation of IRS-1 and PI3-kinase can be impaired by up to 55% by TNFα (del Aguila et al., 1999). A follow up study showed that after 30 minutes of downhill running the insulin transduction signal of IRS-1, PI3-kinase and Akt-kinase, also known as protein kinase B (PKB), was impaired. The production of TNFα was significantly correlated with decreased PI3-kinase activity (del Aguila et al., 2000). These investigations led to the hypothesis that it is a result of

an acute phase response, mediated by TNFα, which is released from inflammatory cells in response to disruptions of the cellular integrity of the muscle cell (Kirwan and del Aguila, 2003).

There is evidence from marathon events that the inflammatory response does not always occur when exercise induced muscle damage is evident (Tuominen et al., 1996, Warhol et al., 1985). This may be a protective mechanism that works to maintain a euglycaemic state during prolonged exercise during glycogen depleting conditions in the exercising muscle (Steinacker et al., 2004). It could be considered that decreased insulin sensitivity is a consequence of EEIMD, the body may be preventing the uptake of glucose into damaged, not fully functioning muscle fibres and therefore sparing it for the intact functional fibres.

2.1.3.2 *Glycogen and exercise*

2.1.3.2.1 General exercise

Bergström et al. (1967) and others (Hermansen et al., 1967) demonstrated the ability to sustain strenuous exercise of a prolonged nature was dependent on the pre-exercise glycogen content of the muscle to be exercised. This was primarily because higher work intensities are reliant upon larger, glycolytic motor units (Armstrong et al., 1974). However, it has also been shown that high levels of intramuscular glycogen are strongly associated with its preferential utilisation during exercise (Bergström et al., 1967, Hargreaves et al., 1995). As the rate of glycogen utilisation increases with exercise intensity (Hermansen et al., 1967), which is closely linked to the metabolic demands of the working muscle, localised muscular fatigue coincides with glycogen depletion (Bosch et al., 1994, Holloszy et al., 1998).

There is a paucity of information on the metabolic demands of resistance exercise, which is likely due to the methodological difficulties associated with the non-steady state conditions of this type of exercise. However, it has been shown that following 30 minutes of intense and prolonged heavy-resistance exercise, glycogen concentrations decreased from 160 to 118 $mmol.kg^{-1}$w.w. (Tesch et al., 1986). This shows that repeated muscular contractions, which are especially strenuous in nature, will reduce muscular stores of glycogen. This could occur within an acute exercise session, which

would reduce both type I and II muscle fibre glycogen content, with the most pronounced changes located in the type II fibres (Koopman et al., 2006).

2.1.3.2.2 Eccentric exercise

When it comes to comparing concentric and eccentric exercise, metabolic studies of human muscle indicate that eccentric activity uses significantly more muscle glycogen than concentric activity at similar intensities (O' Reilly et al., 1987) and impairs glycogen re-synthesis post-exercise compared to muscle depleted by concentric activity (Costill et al., 1990, Doyle et al., 1993, O' Reilly et al., 1987, Widrick et al., 1992).

Costill and colleagues (1990) have shown that feeding with a high carbohydrate (CHO) diet ($8.5g.kg^{-1}.day^{-1}$) for 3 days following EEIMD improved glycogen storage compared to a low CHO diet ($4.3g.kg^{-1}.day^{-1}$). It was also shown that regardless of dietary intervention (high CHO vs. low CHO) a control leg that had performed concentric exercise only had greater glycogen storage than the leg that had completed eccentric exercise. The study also showed that there was no difference in glycogen storage between type I or type II fibres at 24 hours post exercise or across the 72 hours.

Initial rates of glucose uptake in previously eccentrically exercised muscle have been shown to be similar to that of control muscle (concentrically exercised glycogen deplete muscle) in the initial 6 hours post exercise when fed a recovery diet containing a CHO content of $7g.kg^{-1}.day^{-1}$ over 3 days (Widrick et al., 1992). However, this same study showed that accumulation of muscle glycogen slowed over the next 18 hours, such that by 24 hours of recovery, the eccentrically exercised muscle contained 15% less glycogen than the non-exercised control muscle. At 72 hours this difference had increased to 24%. Although eccentric exercise reduces the rate of muscle glycogen accumulation, impairment becomes more apparent at ~26 hours post eccentric exercise.

Asp et al. (1998) showed that during exercise at a constant workload 48 hours following an eccentric bout of exercise, skeletal muscle glycogen utilisation was significantly higher in the leg that had been eccentrically exercised in comparison to a leg that had done no prior exercise.

Eccentric exercise causes a decrease in the muscles ability to store glycogen and subsequently causes an adverse effect on its performance. Therefore, any muscle that

has been exposed to EEIMD will work at higher relative workload and in the process, increase its utilisation of muscle glycogen stores that are already diminished, thereby decreasing its capacity for endurance exercise.

It has been proposed that the reduced rate of glycogen storage observed in previously eccentrically exercised muscles may be the result of competition between inflammatory processes and muscle cells for the available glucose. This brings about an increase in reliance upon glycolysis. The presence of inflammatory cells increases glucose utilisation and lactate production in the muscle (Shearer et al., 1988). These previous studies have suggested that ~70% of the glucose uptake and 50% of the lactate production can be accounted for by the cellular infiltrate within damaged skeletal muscle. Part of this increased glucose uptake by the muscle can be attributed to the oxidation of glucose by the inflammatory cells present in the injured region of the muscle (Forster et al., 1989). Shearer et al. (1988) have shown that inflammatory cells release a soluble factor(s) that can increase glucose metabolism in skeletal muscle by at least 118%, with a 147% increase in the conversion of glucose to lactate.

The reduced ability to restore muscle glycogen during recovery from EEIMD, combined with its higher rate of utilisation when in a damaged state, may be a contributing factor to decreases in performance of exercise. This impact on performance may be of importance for athletes that are exposed to exercise that could induce EEIMD. This may force the muscle to utilise different substrates that are stored within the muscle tissue.

2.1.3.3 Intramyocellular lipid

The majority of the body's energy reserves are stored as fat, mainly deposited as triacylglycerol (TG) in subcutaneous and deep visceral adipose tissue, which has been termed extramyocellular lipid (EMCL). Smaller quantities of TG are present as lipid droplets within the cells of other tissues, notably in the cytoplasm of muscle cells, and are known as intramyocellular lipid (IMCL). Exercise can induce lipolysis in IMCL via the lypolytic enzyme, hormone-sensitive lipase (HSL), resulting in the release of free fatty acids and glycerol. In the case of IMCL, free fatty acids are readily available for oxidation because lipid droplets are usually located in close contact to muscle mitochondria (Boesch et al., 1997).

All fatty acids released from IMCL into the cytoplasm for oxidation are transported to the surface of the outer mitochondria membrane. The fatty acids are then activated via binding with coenzyme A (CoA), converted to a fatty acyl carnitine compound and moved across the mitochondrial membranes while bound to carnitine. Inside the mitochondria, the carnitine is removed, the CoA is rebound and the fatty acyl-CoA molecules are metabolised in the β-oxidation pathway, along with the production of reducing equivalents nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide ($FADH_2$) and acetyl-CoA. The acetyl-CoA is further metabolised in the tricarboxylic pathway with the production of additional reducing equivalents. The electron transport chain, including O_2, accepts the reducing equivalents to generate the proton motive force, which provides the chemical energy used to synthesise ATP from P_i and ADP in the process of oxidative phosphorylation.

The amount of IMCL found between different fibre types and between muscle groups is variable. By dissecting individual muscle fibres out of healthy participants stained for myofibrillar ATPase, Essén and colleagues (1975) showed that type I muscle fibres contained greater amounts of IMCL compared with type II fibres (207 ± 86 vs. 74 ± 46 mmol/kg dry mass (dm) respectively). The large variability in IMCL concentration between muscle groups (Boesch et al., 1997, Rico-Sanz et al., 1998) reflects the fibre type distribution of these muscles. For example, the oxidative type I muscle fibres can contain up to three times more IMCL than the glycolytic type II muscle fibres (Essén et al., 1975, Howald et al., 1985, van Loon et al., 2003). It has been reported that resting IMCL values, obtained from mixed human skeletal muscle samples, can range from 2 to 50 mmol/kg dm (Table 2.1).

Author	Population	IMCL concentration (mmol/kg dm)
(Jansson and Kaijser, 1982)	9 trained males 11 active females	50.4 ± 7.4
(Starling et al., 1997)	7 trained males	37.0 ± 2.1
(Kiens and Richter, 1998)	8 trained males	49.0 ± 5.0
(Bergman et al., 1999)	9 untrained males	2.3 ± 0.5
(Coyle et al., 2001)	7 trained males	28.3 ± 3.6
(Zderic et al., 2004)	6 trained males	36.8 ± 4.8

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

2.1.3.3.1 Measurement

Using biopsy-derived muscle samples, electron microscopy allows identification of the intrafibrillar lipid droplets. Using this technique, the variability of the IMCL in human skeletal muscle has been examined for participants at rest and post-exercise (Howald et al., 1985, Koopman et al., 2006, Wendling et al., 1996), as well as between athletes and non-athletes (Hoppeler et al., 1985). A validation study suggests that IMCL stores measured in repeated biopsies of human skeletal muscle are variable, with a coefficient of variance (CV) of 20 to 26% (Wendling et al., 1996). The within-biopsy variability appears low 6% (the technical error associated with biochemical analysis of the sample). Because of contamination with adipose tissue, despite precautions, between-biopsy variability at rest (19.8 ± 7.9%) and exercise (26.1 ± 17.4%) is higher. Therefore, due to this high variability, only changes that are greater than or similar to 24% of resting IMCL content could be considered meaningful when using the biopsy/biochemical analysis approach for intervention studies.

Biochemical extraction of muscle samples to determine IMCL content has focused on the change in concentration from resting levels to those following acute bouts of exercise. A particular focus has been placed on endurance exercise and these studies are often following some form of dietary or training intervention (Carlson et al., 1971, Fröberg and Mossfeldt, 1971, Phillips et al., 1996b). There is a paucity of research

involving the utilisation of IMCL stores during intense, short-duration or intermittent exercise such as resistance exercise. Following intermittent exhaustive exercise of 30 second bouts of resistance exercise comprising of 6-12 repetitions that were interspersed with 60 seconds recovery for 30 minutes, a decrease in TG content from 23.9 ± 4.6 to 16.7 ± 2.1 mmol/kg dm has been shown (Essén-Gustavsson and Tesch, 1990). The same study also reported that participants with higher resting concentrations of TG showed greater reductions following the exercise protocol. This may indicate that if muscle TG stores are available they will be utilised regardless of exercise intensity, thus providing a glycogen-sparing effect.

However, analysis of changes in lipid content between muscle fibre types has been shown to be different. An acute bout of resistance training incorporating both concentric and eccentric contractions has been shown to bring about a 27 ± 7% reduction in the lipid content of type I muscle fibres of the leg musculature. However, no changes were observed in the type II fibres (Koopman et al., 2006). This study also showed that IMCL accumulation at 120 minutes post exercise had returned to pre exercise levels in all muscle types. This may be attributed to the increase of circulating plasma FFA concentrations, to above pre exercise levels, during the recovery phase post exercise. As exercise continues, plasma FFA rises progressively and promotes uptake and utilisation. Changes in plasma glycerol levels during exercise are suggested to reflect changes in the rate of adipose tissue lipolysis. Any observed increase in plasma glycerol during and after exercise may reflect a continuous FFA release to blood, from where it is subsequently taken up and oxidised by skeletal muscle. This is in line with reports of IMCL accumulation under conditions of elevated plasma FFA, such as prolonged endurance exercise in the non-exercised skeletal muscle tissue (Schrauwen-Hinderling et al., 2003).

The Koopman (2006) study involved working the skeletal muscle against resistance, performing both concentric and eccentric work. No clear indication has been shown on the effects of purely eccentric exercise to stimulate lipolysis during exercise and thereby increase circulating FFA's for oxidisation or re-esterification in recovery from this modality of exercise.

2.1.3.3.2 IMCL and insulin sensitivity

Evidence suggests that an accumulation of lipid within skeletal muscle is associated with insulin resistance (Krssak et al., 1999, Phillips et al., 1996a). Skeletal muscle that is resistant to insulin can be characterised by lower oxidative capacity (Simoneau et al., 1995) and reductions in post-absorptive rates of fatty acid oxidation (Kelley et al., 1999). Skeletal muscle that is insulin resistant has also demonstrated elevated concentrations of lipid, which may be linked to impaired oxidative capacity (Simoneau et al., 1995) and lower rates of fatty acid oxidation by the muscle (Kelley et al., 1999). The possibility has been raised that associations between lipid accumulation within skeletal muscle and insulin resistance is influenced by the muscle's lower capacity to oxidise lipid as an energy substrate. Regular training, of an endurance nature, has been shown to enhance insulin sensitivity (Boulé et al., 2005, DeFronzo et al., 1987, Perseghin et al., 1996) and the capacity for lipid oxidation (Martin et al., 1993, Phillips et al., 1996c).

Despite the numerous observations of the association of high IMCL content and skeletal muscle insulin resistance, an "athlete's paradox" has been widely described (Dubé et al., 2008, Goodpaster et al., 2001, Tarnopolsky et al., 2007, van Loon et al., 2004) in which highly insulin-sensitive, endurance-trained athletes have IMCL content similar to that observed in insulin-resistant obese and type II diabetic subjects. Therefore, IMCL may not confer insulin resistance but rather, any increases in IMCL content would provide substrate for energy metabolism in the exercise-trained state (van Loon, 2004).

The higher IMCL content in the trained state raises the possibility that IMCL is merely a surrogate for other factors within muscle that may directly affect insulin-stimulated glucose metabolism. It has been reported that long chain fatty acyl CoA in skeletal muscle may actually induce insulin resistance by inhibiting hexokinase activity (Thompson and Cooney, 2000). Others have shown evidence that long chain CoA and diacylglycerol (both metabolites of IMCL metabolism) influence insulin signalling pathways in muscle (Itani et al., 2002). Therefore, no direct effect of IMCL on insulin-stimulated glucose metabolism has been clearly defined. With the periodic turnover of IMCL and its associated metabolites resulting from regular exercise may limit their negative influence on insulin signalling.

It has already been established that EEIMD causes instances of insulin resistance in skeletal muscle, but the role played by IMCL in this has not been established. Therefore, the question as to whether IMCL has an influence in insulin resistance that follows EEIMD is an interesting mechanism to explore.

2.1.3.4 High energy phosphates

Phosphocreatine (PCr) serves as an intracellular high energy phosphate pool to replenish sarcoplasmic ATP during high-intensity muscular contractions. This process requires CK to catalyse the phosphotransfer between ATP and PCr (Bessman and Carpenter, 1985). EEIMD results in an increase in the release of CK from skeletal muscle into the circulating bloodstream (for explanation see section 2.1.1) (Clarkson et al., 1986). Theoretically, a loss of CK in skeletal muscle could result in the alteration of the phosphotransfer between ATP and PCr and therefore a reduction in the ability of the muscle to generate force.

Analysis of biopsy-derived samples has been the traditional method of measuring high energy phosphates in EEIMD skeletal muscle. Although chemical changes occur as soon as the tissue sample has been excised from the muscle, to minimise any changes in high energy phosphates, the sample is placed directly into liquid nitrogen. Samples are usually then freeze-dried to allow for non-muscle cell elements such as connective tissue, fat and blood to be removed. This reduces any possible contamination to the sample.

Concerns have been raised over the accuracy of the use of biopsy samples as normative values as there has been wide variation in reported values of resting phosphagen concentration from the quadriceps femoris muscles (mainly utilizing the *vastus lateralis*) (Bogdanis et al., 1996, Hargreaves et al., 1998, Hultman et al., 1996, McKenna et al., 1999) (Table 2.2).

Author	Population	PCr concentration (mM)
(Bogdanis et al., 1996)	8 active males	26.3 ± 1.5
(Hultman et al., 1996)	6 active males	28.1 ± 1.3
	9 active males	26.8 ± 0.5
(Hargreaves et al., 1998)	6 active males	31.7 ± 1.3
(Harris et al., 1974)	81 males	26.4 ± 2.7

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

The variations in values have been attributed to the process of excising the muscle through the biopsy procedure. The theory behind this attribution is that Ca^{2+} activates actomyosin ATPase, which in turn results in the formation of ADP from the hydrolysis of ATP and the restoration of ADP back to ATP at the expense of PCr. It has also been discussed whether or not the time taken to obtain the biopsy and freeze it is sufficient to induce changes in the high energy phosphate concentrations and also perhaps those of other compounds. It has been shown that a one minute delay in freezing a sample can lead to an increase in the apparent [PCr], in the absence of a decrement in the [ATP] of the sample (Söderlund and Hultman, 1986). Further, the change in PCr was not attributable to a readjustment of the CK equilibrium due to increases in pH. The authors concluded that their results showed a partial restoration of PCr (lost as a consequence of damage incurred whilst excising the tissue) through the ability to pump back Ca^{2+} that had been liberated whilst excising the sample into the SR.

A variety of remedial procedures have been implemented to ameliorate the aforementioned problems, though it is still difficult to compare absolute values reported by different research groups. One method to circumvent this issue has been for investigators to take serial biopsies, before and after an intervention. In these instances total creatine (TCr), where TCr = PCr + Cr, has proved itself to be useful as a reference value. However, there are cautions against using TCr to compare between subjects because of the variability that lies within each individual (Harris and Hultman, 1992).

Following eccentric exercise, increases in intracellular [P_i] can inhibit force production via direct action on cross-bridge formation (Westerblad et al., 2002), or at sites in the excitation-contraction pathway. Increases in resting [P_i] and P_i / 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., 2008, Pathare et al., 2005a). Therefore, the reduction in peak torque observed with EEIMD may be related to the increased [P_i] and ensuing alterations in P_i / PCr.

2.2 Magnetic resonance spectroscopy

The previous section examined studies in which markers of change were analysed from muscle extracted using the biopsy technique. This section will review the non-invasive technique of magnetic resonance spectroscopy (MRS) as an *in vivo* method of investigating the effects of exercise and, more importantly, eccentric exercise on skeletal muscle metabolism.

Magnetic resonance spectroscopy is based on fact that different atomic nuclei within tissues can be measured because they behave differently in the presence of an external magnetic field (B_0). MRS has been used to identify nuclei such as hydrogen (H), carbon (C), sodium (Na) and phosphorus (P). When placed in the B_0 of a MR scanner, the direction of spin of tissue metabolites tends to align either with or against the external field. By interspersing a radiofrequency (RF) pulse, the initial orientation is momentarily disturbed causing the nuclei to alter their 'spin'. As the spins flip between alignment and misalignment with B_0, they absorb and radiate energy. Detection of different metabolites of the same nuclei is based on the differences in frequency of spin excited by this RF pulse. Small differences in the frequency between metabolites arise because of their structure and chemical environment. The strength of the signal from the tissue reflects the number of nuclei and is used to derive content of a metabolite within a tissue.

The advantage of MRS over traditional biochemical methods is that it is non-invasive and also offers the possibility of repeated measurements (high time resolution). In contrast, obtaining a muscle sample via biopsy entails a minor surgical procedure that carries risk, is unpleasant to participants and problematic for studies requiring several time points to be studied. An additional advantage over biopsy methods, which are performed on a different tissue sample at each data point, is that with MRS can repeatedly study the same tissue sample provided the spatial relationship between the transreceiver probe and the muscle remains constant (Sapega et al., 1987). Further, a larger tissue sample in both unlocalised and localised (voxel) spectroscopy can be examined during acquisition, therefore the volume of tissue from which metabolites are measured is significantly greater than that permissible with biopsy. This allows for:

different sites of the same muscle, or a number of muscles to be examined simultaneously; metabolite data from the deep musculature that is often too difficult to access by biopsy to be acquired; direct quantification of muscle metabolites allowing for a detailed scrutiny of the cellular components of mitochondrial metabolism negating the reliance on global and/or invasive measures of oxidative phosphorylation (for example $\dot{V}O_{2max}$ measures); determination of real time in vivo biological processes with a temporal resolution in the order of seconds; the ability to obtain longitudinal measurements of skeletal muscle bioenergetics thereby permitting the study of disease progression over time and reliable quantification of the effects of therapeutic interventions.

Skeletal muscle was the first biological tissue that was studied using MRS (Odeblad and Lindstrom, 1955), with the majority of research involving the biochemical pathways that supply energy to the muscle. Application of the technique was initially restricted to cell cultures and biological systems *in situ*, but now MRS is widely used for *in vivo* metabolite measurements in both animal and human muscle.

2.2.1 Intramyocellular lipid

In recent years, a variety of studies have utilised spectroscopy to quantify muscle intracellular lipid concentrations (both IMCL and EMCL) and possible associations between IMCL and insulin resistance (Boesch, 2007, Machann et al., 2004, Schick et al., 1993).

The spectra from proton magnetic resonance spectroscopy (^{1}H-MRS) of skeletal muscle are dominated by water and have significantly smaller lipid resonances, which are observed between 0.9 and 2.5 parts per million (ppm). Using ^{1}H-MRS to measure lipid concentration in skeletal muscle is complex due to the presence of two pools of lipids: IMCL and EMCL. There is a 0.2 ppm separation between the EMCL and IMCL resonances, which is small but allows separate detection of the two pools (Hwang et al., 2001, Schick et al., 1993). The methylene fatty acid protons that comprise the triglycerides of IMCL lipid droplets have been found to resonate at 1.28 ppm and can be differentiated from the signal of EMCL (1.50 ppm) (Boesch et al., 1997, Szczepaniak et al., 1999). This can be shown in Figure 2.3, which shows the components of a typical

^{1}H-MRS spectrum from human skeletal muscle. Two main findings support the interpretation of IMCL and EMCL resonances at 1.28 and 1.5 ppm respectively (Boesch et al., 1997). Firstly, two distinct pools are apparent because one is independent of muscle orientation relative to the B_0 which is consistent within the orientation of triglycerides as droplets in IMCL, whereas the signal from extracellular triglyceride fatty acids is dependent on the orientation with the B_0. This can be explained by the fact that IMCL droplets are evenly distributed in muscle tissue, while EMCL are either bulk fat depots or adipocytes along fasciae. Secondly, when voxel size is increased, IMCL resonances scale with signal amplitudes of metabolites in the muscle cell (e.g. creatine), whereas the lipid signals of bulk (EMCL) fat show a disproportionate growth. Accordingly, if the voxels are not small enough or placed carefully in the site of interest in the muscle, the EMCL signal can dominate the ^{1}H-MRS spectrum.

The IMCL pool is believed to serve as an important source of energy supply during endurance activities (Boesch et al, 1997) as opposed to the EMCL signal which corresponds to adipocytes between the muscle fibres that constitute the long term storage depot for lipids. The two pools are kinetically different because EMCL turns over very slowly whereas there is significant evidence that IMCL is dynamic and its concentration is readily modifiable via dietary or activity changes (Boesch et al., 1999).

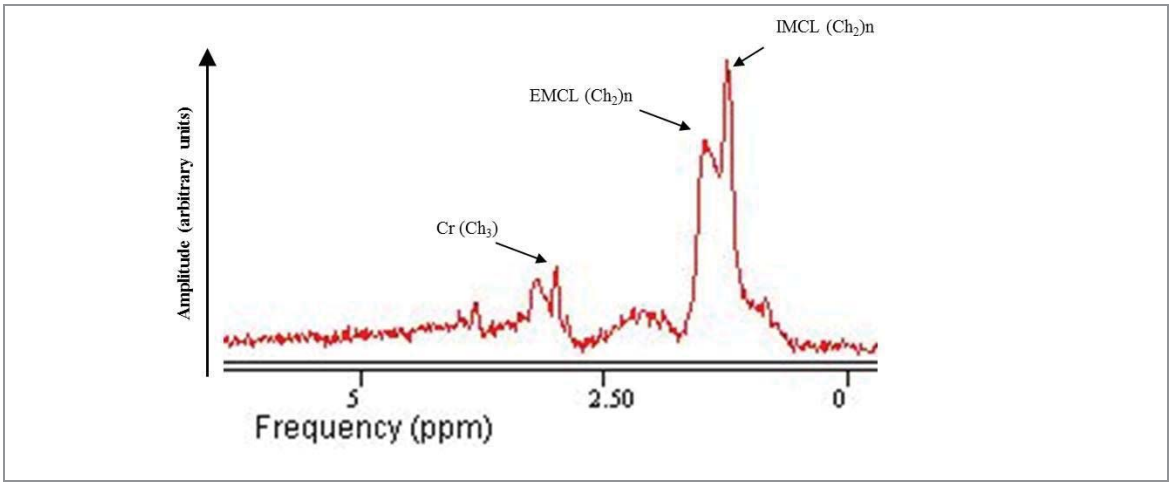

Figure 2.3. Muscle spectrum by ^{1}H-MRS. The figure shows a ^{1}H-MRS spectrum from a human *vastus lateralis* muscle. The arrows point out the peaks originating from the methylene protons of EMCLs and IMCLs and creatine (Cr), respectively. Frequency is expressed as deviation from the frequency of a reference compound and is measured in millionths (ppm, that is parts per million) of the instruments operating frequency.

Measurement of IMCL via ^{1}H-MRS has been found to correlate well with more established methods from biopsy-derived tissue sample, including electron microscopy and histochemistry using Oil Red O staining (Schrauwen-Hinderling et al., 2006, Szczepaniak et al., 1999). The intra-individual CV between three repeated measures obtained from the same trained leg with ^{1}H-MRS was 6.1–16.9%, depending on the internal reference standard (Boesch et al., 1997). Similar values are reported in the literature for multiple muscle biopsy measures (12%) in trained men (Watt et al., 2002), which is in contrast to the large variability (23%) associated with multiple muscle biopsy measures in untrained men (Wendling et al., 1996). The IMCL CV, when applied to obese subjects, was not greater than that reported for non-obese men and women of various training statuses (Steffensen et al., 2002). Non-invasive ^{1}H-MRS technique therefore offers excellent reproducibility, permitting the accurate quantitative assessment of changes in IMCL across a range of populations. This may also transfer to reproducible measures within muscle tissue that may have been disrupted via eccentric based exercise and thus allow accurate measures of IMCL utilisation / storage to be quantified.

The sensitivity of ^{1}H-MRS to changes in lipid content is high and it has proven to be a more reliable for measuring IMCL than both morphometry and histochemical analysis of IMCL (Schrauwen-Hinderling et al., 2006). The accuracy and sensitivity of ^{1}H-MRS in measuring IMCL concentrations is also sufficient to measure changes in IMCL (depletion and recovery) after exercise (Boesch et al., 1999).

2.2.2 High energy phosphates

By allowing the measurement of cellular high energy phosphates, phosphorus magnetic resonance spectroscopy (^{31}P-MRS) enables the study of the bioenergetics of normal and pathological muscle tissue. In a phosphorus spectrum acquired from a skeletal muscle (Figure 2.4), seven peaks are visible at clinically available field strengths (1.5 – 3.0T). The major peaks correspond to P_i, PCr and three phosphate groups of ATP. In addition, peaks from phosphomonoesters (PME) and phosphodiesters (PDE) can be detected at 1.5 - 3.0T but with poorer spectral resolution due to their low tissue content. In addition to being used to quantify metabolite concentrations ^{31}P-MRS has been used to quantify

the metabolic cost of various physiological processes in skeletal muscle, particularly ATP turnover (Kemp and Radda, 1994).

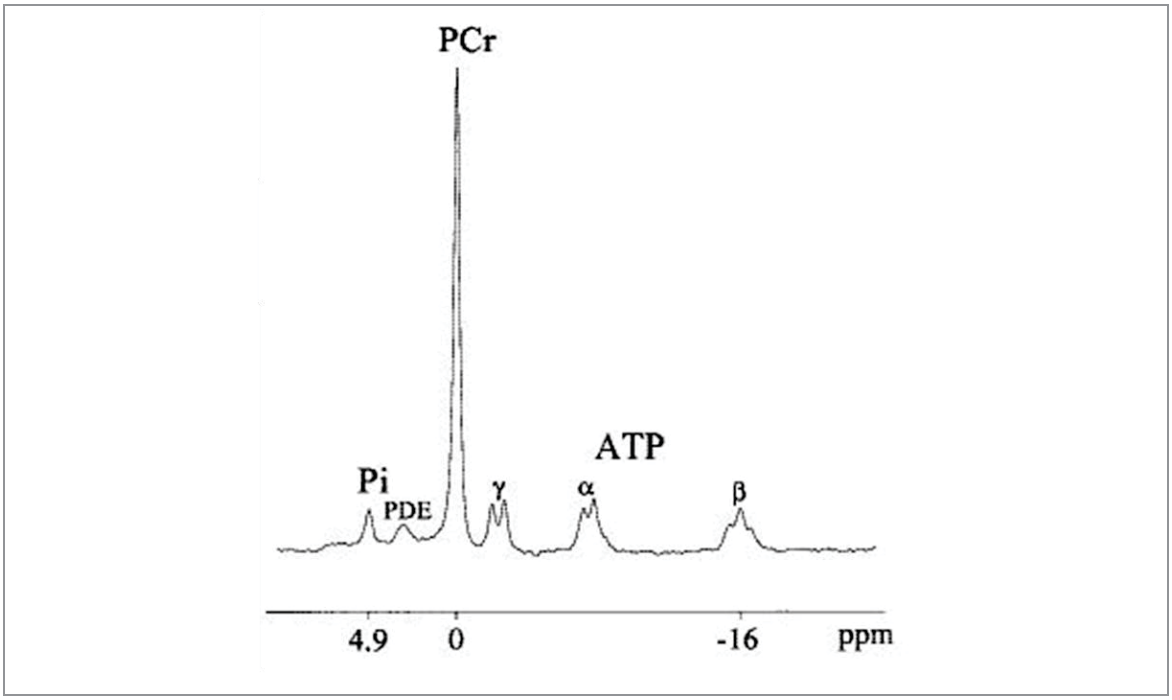

Figure 2.4. A normal resting phosphorus spectrum of muscle. The major peaks of the P-MR spectrum are: phosphocreatine (PCr), inorganic phosphates (P_i), phosphodiesters (PDE), and the three peaks alpha (α), beta (β) and gamma (γ) ATP. The chemical shift in ppm of the P_i is used for calculation of intracellular, cytosolic pH. Adapted from Argov et al. (2000), p.1317.

The main application of ^{31}P-MRS has been to investigate mitochondrial function by measuring the net consumption of ATP in skeletal muscle and the resynthesis of [PCr] during and after muscular work or other metabolic insults (Kemp, 2000, Kemp and Radda, 1994, Kemp et al., 1993, Meyer, 1988). It allows estimations of maximal rates of oxidative ATP synthesis to be compared with *ex-vivo* measures of mitochondrial function (Rasmussen et al., 2001). The high energy phosphates ATP and PCr indicate the status of short-term energy supply within skeletal muscle.

The error variance of MRS for quantification of metabolite concentrations is similar to or less than that encountered with traditional invasive assays (Gadian et al., 1977, Meyer et al., 1986, Meyer et al., 1985, Mole et al., 1985). This is because analytical biochemistry permits measurements of the more stable compounds of metabolism such as ATP, while the more labile compounds are measured with less accuracy because the

best biopsy and assay techniques still allow some breakdown of the unstable high energy phosphates during penetration and analysis (Gadian et al., 1977, Meyer et al., 1985, Meyer et al., 1982, Seraydarian et al., 1961). For example, ^{31}P-MRS has consistently shown a higher value for [PCr] in resting muscle than the values determined by the freeze-clamped biopsy technique (Kemp et al., 2007(Edwards et al., 1982). These variations in concentration are attributed to the rapid hydrolysis of PCr to P_i during the freeze-clamp process. This explanation is supported by observations that the total phosphorus concentration ([PCr + P_i]) determined by either method are the same. The same discrepancy between the two techniques does not appear when measuring [ATP]. This may be because of the maintenance of equilibration of the CK reaction during the excision of tissue during the biopsy.

The ability to examine metabolism of skeletal muscle with ^{31}P-MRS (both at rest and during exercise) has proven valuable. One of the most outstanding features of ^{31}P-MRS is the ability to continuously obtain time dependent metabolic information from living tissues. ^{31}P-MRS acquisition can typically permit metabolite concentration to be determined each 4 – 16 seconds (Kemp et al., 2007). Higher acquisition frequency permits enhanced time resolution, allowing for improved evaluation and quantification of muscle oxidative and glycogenolytic energetics *in vivo* (Argov and Arnold, 2000).

2.2.3 Quantifying metabolites using ^{31}P-MRS

High energy phosphates have been discussed as possible markers of altered metabolic function in skeletal muscle following acute bouts of eccentric exercise. Studies have used the ratio of inorganic phosphate (P_i) and PCr (P_i / PCr) as a marker of muscle damage following eccentric exercise, concluding that an increase in this ratio can reflect non-specific damage in normal muscle (McCully et al., 1988) and may reflect variations in intracellular adenosine diphosphate [ADP]. In healthy skeletal muscle, the [ADP] controls the availability of intracellular free energy, directly determining the rate of mitochondrial ATP synthesis and both [ADP] and [P_i] reflect the rate of oxidative metabolism (Chance et al. 1986). However, the ADP resonance cannot be resolved from the muscle ^{31}P-MRS spectrum and is instead calculated from the P_i / PCr. As such,

P_i / PCr may indicate altered muscle metabolism through an increased resting oxidative flux or a reduced sensitivity of the mitochondria to changes in cellular free energy.

Metabolite concentrations are commonly quantified from relative spectral amplitudes using ATP peaks as an internal standard (i.e. assuming a [ATP] of 8.2mM based on the literature) (Kemp et al., 2007). P_i / PCr ratio is closely related to the phosphorylation potential and reflects the energy state of the muscle (Chance, 1984, Veech et al., 1979). At rest, P_i / PCr ratios are around 0.12 in healthy human muscles, depending upon the muscle studied (Wiener et al., 1986). The position of the P_i peak depends upon the relative concentrations of the two molecules ($H_2PO_4^-$ and H_3PO_4) and, based on frequency shifts of the P_i peak, intracellular pH can be determined. Intracellular pH is calculated from the difference in the chemical shift between PCr and P_i. The relative levels of otherwise undetectable metabolites including ADP and ionic concentrations such as magnesium (Mg^{+2}) can also be indirectly estimated using ^{31}P-MRS based on measurements of Cr, PCr, pH and the equilibrium constants. As discussed below, variations in phosphate metabolite concentrations from rest to exercise and during recovery have been used to illustrate muscle energetics *in vivo*. The phosphorylation potential is the energetic potential of the cell. That is, the phosphorylation potential is high when [PCr] are high and [ADP] are low. Changes in resting metabolite content, metabolite ratios and a change in pH reflect disturbances in the metabolic pathways and/or possible structural dysfunctions of the mitochondria or a diseased state of the muscle as a whole (Heerschap et al., 1999, Mattei et al., 2004). Increases in resting [P_i] and P_i / PCr ratios in skeletal muscle obtained by ^{31}P-MRS have been observed in a variety of disorders including primary mitochondrial diseases, myopathies, muscle injury and disuse (Argov and Arnold, 2000, McCully et al., 1988, Pathare et al., 2008). Moreover, the elevated [P_i] and P_i / PCr ratios were found to be closely inversely associated with the force generating capacity of the skeletal muscle. Furthermore, acidification of the muscle results in the movement of the P_i peak toward the PCr peak. As the resonance detected from the ^{31}P-MRS spectrum predominantly reflects the changes in the muscle phosphates in the cytosol (Gadian, 1992), the determination of pH using the P_i resonance shift reflects the pH of the cytoplasm and is termed intracellular pH.

Even though the contents of the air-equilibrated samples have been considered to be a more accurate state of resting muscle they are still lower in value than those reported in MRS studies. However, MRS-derived measures of substrate concentration are also subject to error. The major argument in finding an error with the ^{31}P-MRS spectra are that these rely on calibration of the ATP and P_i peaks against absolute values, with these values being derived from biopsy samples themselves. Furthermore, concentrations of PCr, ADP and pH are derived from the amplitude and/or chemical shift of these visible peaks on the basis of assumptions, which are often based on biopsy-derived values. Also, there is an assumption that all of the ATP and P_i measured in extracted muscle is visible during MRS, which is often not the case if they are bound to proteins or mitochondria (Takami et al., 1988), which could lead to overestimation of [PCr] in skeletal muscle.

2.2.4 Quantifying the metabolic consequences of EEIMD with ^{31}P-MRS

Early studies that utilised MRS as a tool of measuring metabolic perturbations associated with EEIMD looked at measures of the resting values of P_i and PCr in undamaged muscle versus muscle that had been damaged following a single bout of eccentric exercise (Aldridge et al., 1986, McCully et al., 1988, Rodenburg et al., 1994). These studies indicated that mild forms of muscle damage to either the wrist flexors or soleus resulted in increases in resting P_i / PCr that remained elevated for several days. No increases were shown in undamaged / control muscles that had undertaken similar isometric or concentric work. It was also noted that the levels of P_i / PCr and CK activity in the damaged tissue were comparable to those in patients with various neuromuscular diseases and is suggestive of an increase in resting muscle metabolism following EEIMD (McCully et al., 1988a).

There has been limited investigation of possible alterations to exercising muscle metabolism following EEIMD in humans due to the difficulties in constructing MRI-safe ergometry equipment suitable for use within the scanner. Using a specifically designed hydraulic ergometer for exercise of the quadriceps within a whole body MRS (Rodenburg et al., 1994), participants completed two graded concentric exercise tests

before and 24 hours after performing stepping exercise designed to induce EEIMD (Rodenburg et al., 1995). The expected increase in the resting P_i / PCr ratio was observed but no differences between damaged and undamaged muscle were reported in the P_i / PCr ratio in response to the exercise test. While these findings indicated that exercise metabolism was not altered by the prior 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 EEIMD (Rodenburg et al., 1995)

In summary, many studies of skeletal muscle have been conducted following acute bouts of eccentric exercise. However, there are problems with the differences in the physiological state of these muscles, namely differences in the 'trained' status of the tissue under examination (i.e. previous exposure to eccentric biased exercise). This raises the question of whether exposure to adaptive bouts of eccentric exercise has an effect on the high energy phosphate pools within skeletal muscle.

2.3 Adaptations in response to repeated bouts of eccentric exercise

Following a single, acute bout of eccentric exercise, previous studies have shown evidence of EEIMD. However, following a single bout of eccentric exercise, skeletal muscle can be protected against damage caused by subsequent bouts of eccentric muscle action. This effect is commonly referred to as the repeated bout effect (RBE) (McHugh, 2003, Nosaka and Clarkson, 1995).

2.3.1 Evidence of adaptations

Section 2.1 examined the markers of change in response to acute bouts of eccentric exercise, which included structural and biochemical (enzyme and protein leakage, loss of Ca^{2+} homeostasis, inflammatory response and DOMS) alterations. In muscle experiencing strenuous eccentric exercise following prior bouts of eccentric exercise there is attenuation of the appearance of damage markers, a near absence of weakness and reduction of perceptions of soreness (Brown et al., 1997a, Howatson et al., 2007, Howatson and van Someren, 2007, McHugh et al., 2001, McHugh et al., 1999, Nosaka and Clarkson, 1995, Nosaka and Newton, 2002b, Nosaka et al., 2001).

The RBE has been evidenced to appear as early as 2 days after the initial exercise bout (Nosaka and Newton, 2002b, Paddon-Jones et al., 2000) and is diminished between 9 and 12 months later (Nosaka et al., 2001). Further, the effects of 8 weeks of consecutive eccentric training found that pronounced soreness and muscle fibre disruption was evident only following the first 2 to 3 eccentric training sessions, with marked decreases in these variables during the remainder of the study (Fridén and Lieber, 2001). However, minimal amounts of prior exercise can also confer the effect. As few as 2 maximal eccentric contractions have been shown to offer protection against symptoms of EEIMD when the same muscle group performed a larger amount of maximal eccentric work 2 weeks later (Nosaka and Sakamoto, 2001). It has also been shown that light eccentric exercise which does not induce symptoms of muscle damage would confer a protective effect. Exercise equating to just 10% maximal voluntary

contraction (MVC) is as effective in attenuating the markers of EEIMD from a subsequent bout of eccentric exercise that was greater in magnitude to the initial bout (40% MVC) performed 48 hours later (Lavender and Nosaka, 2008).

Care must be taken when comparing studies that utilise differing protocols because these have provided contrasting timeframes of the RBE. For example, eccentric elbow flexion studies resulted in significantly greater and a more focal degree of muscle injury than downhill treadmill running. Additionally, the fact that most individuals spend more time using their legs for purposes of walking may result in an already present protective effect, which is lacking in elbow extension studies in the less frequently used arms.

The RBE has provided evidence that an initial bout of eccentric exercise offers a protective effect on subsequent bouts of similar exercise type for as long as 6 to 9 months. In most of the studies, the RBE is evidenced by a more rapid return to full force generating capacity, decreased rating of perceived soreness and reduced serum CK activity following repeated versus unaccustomed eccentric muscular activity. Furthermore, the initial bout does not have to be particularly severe to bring about the adaptation. An underlying mechanism responsible for the adaptive process has not been fully revealed, although several have been offered as an explanation.

2.3.2 Theories on mechanisms for adaptive process

There are three theories used to account for the adaptation to an initial bout of eccentric exercise, which can be categorised into neural, connective tissue and cellular theories (McHugh, 2003, McHugh et al., 1999).

The neural theory proposes that the initial damage to skeletal muscle stimulates an increased motor unit activity (Komi and Buskirk, 1972), slow-twitch fibre recruitment and motor unit synchronization following damage (Golden and Dudley, 1992). Subsequently, forces encountered during a repeated bout of eccentric contractions are spread across more fibres (Nosaka and Clarkson, 1995).

The connective tissue theory suggests that adaptive increase in the amount of intramuscular connective tissue (Lapier et al., 1995) and remodelling of the intermediate

filaments (Fridén et al., 1983a) in response to eccentric contractions account for the attenuation of damage and DOMS in response to repeated eccentric work.

Cellular adaptation theories suggest a strengthening of cell membranes (Clarkson and Tremblay, 1988), removal of weak fibres (Armstrong et al., 1983) and longitudinal addition of sarcomeres (Lynn and Morgan, 1994) as likely candidates for the adaptive response to eccentric muscle work. A significant reduction in the levels of serum CK activity measured after a repeated bout of eccentric exercise have been put forward as an indication that the cell membrane has been strengthened and is less likely to allow a loss of Ca^{2+} homeostasis and subsequent necrosis of cellular components (Clarkson and Tremblay, 1988).

In summary, the effect of RBE research shows that adaptation has been produced even when the initial bout is less demanding than the second bout, whether that is by number of contractions performed or the length the muscle is contracting through. The ability for the conferral of the protective effect is not necessarily reliant on damage being evident following the first bout of eccentric exercise. A number of adaptive responses are likely to be involved in the mechanism of protecting the muscle from further damage although there is no unified theory that can explain why this mechanism occurs.

2.4 Aims and objectives

Direct histological analyses of muscle tissue have revealed that unaccustomed, eccentric exercise results in substantial disruption to skeletal muscle structure and function. There has been little examination of the effects of EEIMD on muscle metabolism *in vivo*. This thesis sought to investigate metabolism after vigorous sessions of eccentric and concentric contractions to establish what relationship(s), if any, exist between these and muscle metabolism. In addition, mechanical and biochemical characteristics of exercised muscle were studied to ensure that damage had occurred within the muscle(s) of interest. Several questions were proposed and hypotheses are postulated from a series of studies and these included:

1. Are there changes in substrate selection during concentric exercise when performed following an acute bout of eccentric damaging exercise?
   Hypothesis: Following a bout of acute eccentric exercise measurable changes would be seen in the rate of both CHO and lipid oxidation, with greater reliance on CHO in the EEIMD condition. **(Chapter 3)**

2. If substrate selection is altered following EEIMD, involving lowered lipid oxidation, does that affect the storage of IMCL?
   Hypothesis: The purported alteration in lipid oxidation following exposure to an acute bout of eccentric exercise will increase the storage of intramyocellular lipids (IMCL) in the damaged tissue. **(Chapter 4)**

3. Are high energy phosphate concentrations altered in resting muscle following an acute bout of eccentric exercise?
   Hypothesis: Following an acute bout of eccentric exercise sarcolemma disruption would increase the [Pi] without alteration in [PCr] thereby increasing Pi / PCr ratio. This change in Pi / PCr could therefore be employed as a of muscle damage. **(Chapter 4)**

4. Eccentric exercise has been associated with transient insulin resistance. Does exposure to multiple bouts of eccentric exercise influence insulin sensitivity and

glucose disposal (glycaemic response) to an oral glucose load? Therefore, the purpose of this study was to determine whether the insulin resistance experienced following an acute bout of eccentrically-biased contractions would be diminished following a second bout of eccentric contractions in accordance with the concept of an adaptive repeated bout effect

Hypothesis: That the insulin and glucose responses to an OGTT would be attenuated following a repeated bout of eccentric biased exercise. **(Chapter 5)**

5. Does prior training via differing contraction modalities (eccentric vs. concentric) have an effect on skeletal muscle high energy phosphate concentrations at rest following EEIMD?

   Hypothesis: with the marked increase in [Pi] and Pi / PCr ratio following an acute bout of eccentric exercise these would be attenuated following a period of repeated exposure to eccentric biased exercise. **(Chapter 6)**

3. Indirect measures of substrate utilisation following eccentric exercise induced muscle damage

3.1 Abstract

This study investigated whether eccentric exercise-induced muscle damage (EEIMD) resulted in changes to whole body substrate utilisation during subsequent exercise 48 hours later. Eight males (31 ± 6yrs; mean ± SD) performed 30 minutes of bench stepping exercise (BSE). One leg performed eccentric contractions (Ecc) by lowering the body whilst the control leg performed concentric contractions (Con) by raising the body. On the two days following BSE participants performed measures of muscle function on an isokinetic dynamometer and undertook a bout of one leg cycling exercise, at two differing workloads, with the first workload (WL1) at 1.5 ± 0.25 W/kg and the second workload (WL2) at 1.8 ± 0.25 W/kg with each leg. Expired respiratory gases were collected during cycling to estimate whole body substrate utilisation. There were significant decrements in measures of muscular performance (isometric tension, concentric and eccentric torque) and increased perception of soreness in Ecc compared with Con ($P < 0.05$). The effect of the Ecc treatment on substrate utilisation during one legged cycling revealed a significant trial × time interaction with higher rates of CHO oxidation in the Ecc condition compared with Con that were further increased 48 hours later ($P = 0.021$). A significant treatment × time × effort interaction ($P = 0.005$) indicated the effect of the treatment altered as workload increased with higher rates of CHO oxidation occurring in WL2. This is consistent with greater reliance upon muscle glycogen. This suggests that in EEIMD, reductions in strength and increased feelings of soreness are associated with greater reliance upon intramuscular CHO oxidation, than lipid, during subsequent concentric work.

3.2 Introduction

Glucose uptake by EEIMD skeletal muscle is reduced compared with muscle that has undertaken concentric exercise (Costill et al., 1990, Doyle et al., 1993, O' Reilly et al., 1987, Widrick et al., 1992) and it has been demonstrated that glycogen resynthesis is impaired following EEIMD (Asp et al., 1998, Asp et al., 1995, Costill et al., 1990, Widrick et al., 1992). Pre-exercise availability of intramuscular glycogen and triglycerides largely dictates fuel selection during exercise (Coyle et al., 2001, Johnson et al., 2003a). The available evidence suggests that the mechanical damage brought about as a consequence of eccentric exercise should produce changes in muscle substrate utilisation during subsequent exercise.

Previous studies investigating the effect of prior damage on substrate utilisation during subsequent exercise 48 hours later (Asp et al., 1998, Widrick et al., 1992) have compared the damaged muscle to a non-exercised control. However, as intramuscular substrate concentrations are heavily influenced by prior exercise, these studies were unable to provide a complete understanding of the effect of prior eccentric exercise on subsequent substrate utilisation. Thorough elucidation of the effect of EEIMD on muscle metabolism necessitates that the control muscle complete the same amount of external work but in a concentric fashion.

The purpose of this study was to test the hypothesis that EEIMD produces changes to the substrate utilisation of skeletal muscle that is undertaking a subsequent bout of concentric exercise. To test the hypothesis, 8 men performed Ecc of one leg, whilst completing an equivalent amount of work, concentrically, with the other leg. At 24 and 48 hours after this exercise, respiratory gases were collected during bouts of one legged cycling and substrate utilisation calculated in each leg. Employing a one legged model means that circulating factors will affect legs equally and thus the concentric leg acts as a control.

3.3 Methodology

Participants

Eight males (mean ± SD; age, 31 ± 6 years; height, 179.5 ± 5.1 cm; $\dot{V}O_{2max}$, 67.4 ± 8.9 $mL \cdot kg^{-1} \cdot min^{-1}$ and body mass 75.2 ± 6.2 kg) who were familiar with resistance training but had not been exposed to eccentrically based exercise in the six months preceding the study volunteered to participate in this research. None of the participants had a history of muscle or metabolic disease. All participants completed a medical screening questionnaire and provided written informed consent. Participants were asked to refrain from strenuous exercise for the preceding two weeks and for the duration of the study intervention. The study was performed according to the Declaration of Helsinki and approved by the University's Research Ethics Committee prior to the start of the investigation.

Experimental overview

Participants reported to the laboratory on six occasions. During the first three visits they were familiarised with the one leg cycling protocol. The following three visits formed the experimental sessions, with the first of these visits consisting of baseline measures and the eccentric exercise. The eccentric protocol consisted of 30min of bench stepping exercise (BSE) with an additional 10kg mass. The study was conducted in a randomised balanced design. Each participant was randomly assigned a leg that would undertake the eccentric work (Ecc), with the other leg serving as their concentric control (Con). For all participants each bout of one legged cycling was conducted with the right leg undertaking the measures first. On each experimental visit to the laboratory participants reported after an overnight fast and measures of muscular performance (isometric tension, concentric and eccentric torque), perceived muscular soreness, capillary blood creatine kinase (CK) activity, whole body oxygen (O_2) uptake, carbon dioxide (CO_2) production, respiratory exchange ratio (RER), blood lactate concentration and rating of perceived exertion (RPE) were measured.

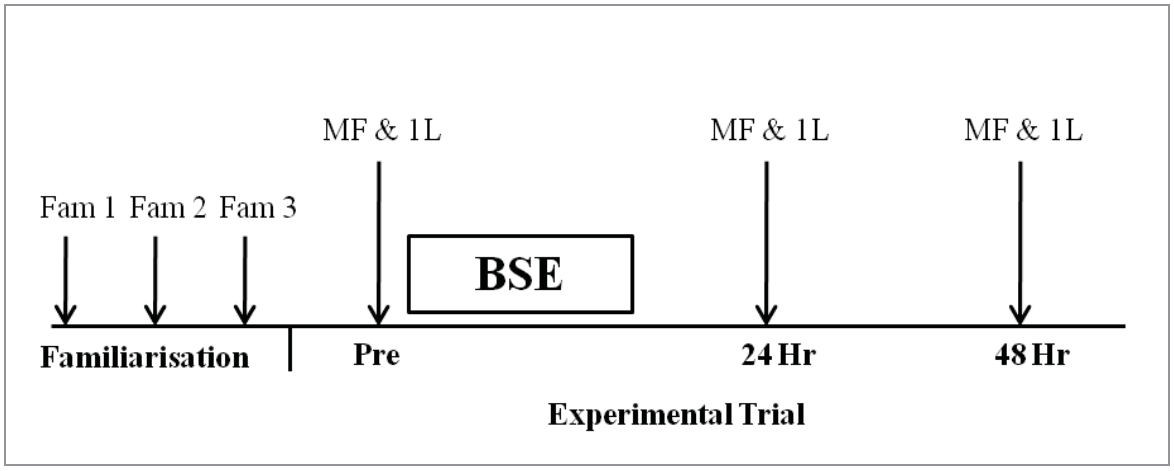

Figure 3.1. Schematic presentation of the experimental protocol. Indicating, familiarisation sessions (Fam1, Fam 2 and Fam 3), measures of muscle function (MF) and one legged cycling (1L) that were conducted pre bench stepping exercise (BSE) and 24 and 48 hours post BSE.

In the 48 hour period preceding the study, subjects maintained a diet comprising a balanced macro-nutrient intake, which was recorded through use of weighed food diaries, whilst maintaining a constant activity level where cycling was allowed. Each subject attended the laboratory at 24 and 48 hours after the eccentric exercise protocol for further testing (as described below). On each occasion, attendance was in the overnight-fasted state. The diet was consumed until after the 48 hour tests and the participants were instructed to avoid alcohol and smoking during the entire experimental period. After each day of trials the participants were given a standardised breakfast to control for substrate availability. The breakfast equated to 2020 kilojoules (kJ) and comprised 63% CHO, 22% fat and 15% protein. Participants abstained from any strength based exercise for a two week period prior to and for the duration of the study.

Eccentric exercise protocol

Participants performed a single bout of 30min of bench-stepping at a frequency of 15 cycles per minutes at a predetermined step height of 110% of their lower leg length, as detailed elsewhere (Newham et al., 1986, Newham et al., 1983a). Stepping up with one leg ensured that the muscles that are required for knee and hip flexion were working concentrically. Stepping down from the bench with the same leg first then requires the muscles of the contralateral leg to contract eccentrically. The leg that performed the

eccentric action was randomly chosen with 4 participants performing eccentric actions with the left leg and 4 participants performing eccentric action with the right leg. All participants performed the stepping exercise wearing a vest (Speed Power and Stability Systems Ltd, Christchurch, New Zealand) containing 10kg of additional mass, which equated to a mean of 13 ± 1% of their body mass. This protocol was utilised to induce muscle damage as it would expose a larger number of muscle groups to eccentric work compared to the use of an isokinetic dynamometer and would maximise the damage to musculature of the lower limb that are predominant in the pedal action of cycling.

Muscle performance

Participants were seated on a Biodex® isokinetic dynamometer (Biodex Medical Systems, New York, USA) and straps were fixed across the chest, hips and active leg to isolate movement to the quadriceps. Knee joint range of motion (ROM) was set and recorded for use in subsequent tests. Participants performed five maximal isometric, concentric and eccentric contractions of the quadriceps muscles of both the Ecc and the Con legs. Isometric tension was measured at a knee angle of 75° (1.31 rad). Concentric and eccentric torque was measured at an angular velocity of $30°s^{-1}$ ($0.52\ rad.s^{-1}$). Range of motion was determined whilst seated on the dynamometer at maximal knee flexion (0°). Participants were instructed to extend their knee through a ROM of 60° (1.05 rad). Absolute peak torque over five contractions was recorded. Each set was separated by 2 minutes of passive recovery.

One legged cycling exercise

All cycling bouts were conducted on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands). The cycling session consisted of cycling with one leg for two 5 minute stages of fixed intensity. Participants were warmed up by cycling for 5 minutes with both legs simultaneously at 100W, then a further 5 minutes with one leg at a workload of 1.25 ± 0.27 W/kg. This 5 minutes of one legged cycling allowed for the leg to become accustomed to the increase in blood flow to the working muscles. Participants then cycled for a period of 10 minutes with their right leg first. During this 10 minute period the workloads were fixed and increased after 5 minutes. The workload was determined during familiarisation sessions and expressed as power to body mass ratio (W/kg). The workloads were 1.5 ± 0.25 W/kg (WL1) and 1.8 ± 0.26

W/kg (WL2). The workloads varied from 88W to 166W (114 ± 18W, 135 ± 19W across the two workloads). The specific intensities selected were based on pilot work and shown to elicit an increase in heart rate (HR), which was monitored continuously (Polar FS1, Polar Electro Oy, Finland), as well as RPE using a Borg scale during the exercise bout.

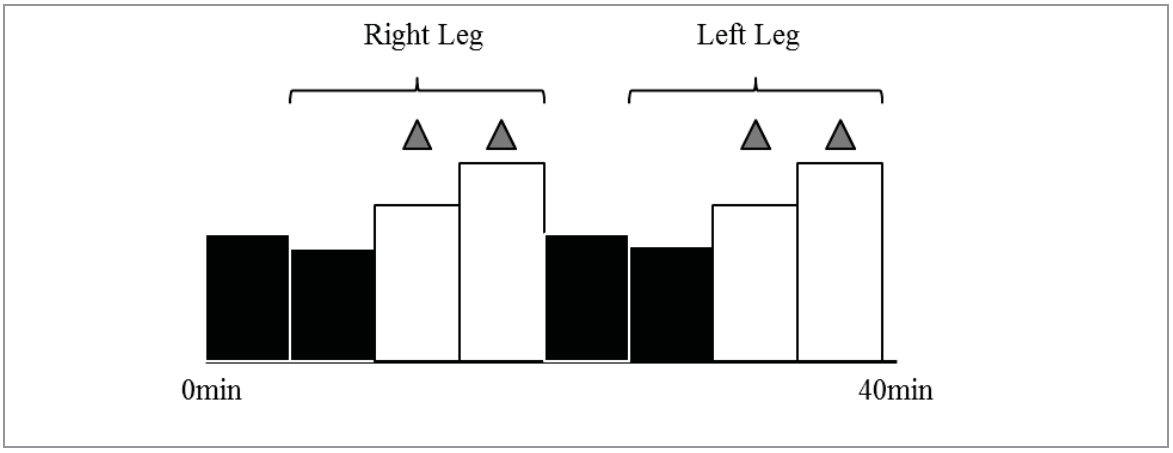

Figure 3.2. Schematic presentation of the one legged cycling protocol. Black rectangles represent the warm up cycling (two and one legged cycling). White rectangles represent fixed one legged cycle stages. Triangles represent all measures collected during cycling.

During the one-legged cycling, exhaled air was collected using Douglas bags during the final two minutes of the last two 5 minutes stages and measured with a Ametek analyser (AEI Technologies, Pittsburgh, PA) and dry gas meter (Harvard Apparatus, UK) to determine O_2, CO_2 and RER. Ambient temperature and barometric pressure were measured before each ride and relative humidity calculated from the wet and dry bulb thermometer differential. During the final 10s of each stage, RPE was assessed using a Borg scale (Borg, 1970).

Substrate utilisation during the post eccentric exercise one legged cycling was calculated after each trial using the stochiometric equations of Frayn (Frayn, 1983), where oxidation of CHOs is given by the equation:

$$CHO = 4.55 \times \dot{V}\,CO_2 - 3.21 \times \dot{V}\,O_2 - 2.87n$$

and the oxidation of fat is given by the equation:

$$Fat = 1.67 \times \dot{V}O_2 - 1.67 \times \dot{V}CO_2 - 1.92n$$

the nitrogen excretion rate (n) was assumed to be 135 $\mu g \cdot kg^{-1} \cdot minute^{-1}$ (Romijn et al., 1993).

Blood sampling

Approximately 30 µL of capillary blood was collected from a finger via a prick made with a spring-loaded lancet. The blood sample was immediately analysed using a Reflotron® systems spectrophotometer (F.Hoffman-La Roche Ltd, Basel, Switzerland) for plasma CK activity. The normal reference range for CK using this method is 50 to 220 $IU \cdot L^{-1}$ and the assay can accurately detect values between 20 and 2000 $IU \cdot L^{-1}$, according to the manufacturers' manual.

Capillary blood lactate concentration was collected from a finger via a prick made with a spring-loaded lancet prior to and at the end of each workload during each leg one-legged cycling exercise bout. The sample was analysed for blood lactate concentration using an automated lactate analyser (Lactate Pro™ Arkray TM, Kyoto, Japan), which was calibrated and operated in accordance with the manufacturer's instructions.

Perceived muscle soreness

Using a visual analogue scale (VAS) participants gave an indication of their current level of perceived muscle soreness on a subjective scale. The scale was 10 cm in length, with 0 (no soreness) and 10 (very, very sore) representing the ends of the scale (Cleak and Eston, 1992b). Measures were taken prior to the eccentric exercise protocol and then 24hours and 48hours post eccentric exercise. Soreness was rated while stepping up (Con) onto the box used during the eccentric exercise protocol and lowering off the box (Ecc) of the loaded quadriceps muscle. Both legs were assessed for ratings of perceived soreness in both contraction types.

Statistical analyses

All statistical analyses were performed using Predictive Analytics SoftWare (PASW) Statistics 18 for Windows (SPSS Inc., Chicago, IL, USA). All data are reported as the mean ± SD. Data was tested for approximation to a normal distribution: if residuals

were considered not to be normally distributed, data were log-transformed and residuals were investigated again. After log-transformation, residuals were considered to be normally distributed and thus for these measurements log-transformed data were used in the subsequent statistical analysis. Baseline (pre-intervention) respiratory gas data were compared between legs using a two-way analysis of variance (ANOVA) (treatment × workload) to ensure there were no initial differences. Subsequently, respiratory gas, blood and HR and RPE data were analysed using a three-way (treatment × time × workload) ANOVA for repeated measures. Muscle function and perceived soreness data were compared using a two-way ANOVA. Values from ANOVA were assessed for sphericity. Only treatment × time and treatment × time × workload are reported in the results as these are relevant to the hypotheses. On discovery of a significant F test, pair-wise comparisons were identified using Tukey's honestly significant difference (HSD) post hoc procedure. Measures of CK activity were analysed using a one-way repeated measures ANOVA. Statistical significance was accepted at P values $\leq$ to 0.05.

Results

Baseline comparisons

There were no significant differences between legs for any dependent variable of interest prior to the intervention. Significant treatment × time and treatment × time × workload interactions reported below thus reflect effects of the treatment and effects of the treatment as they interact with workload.

Skeletal muscle function

There were significant treatment × time interactions for all measures of muscle function ($P = 0.006$; $P = 0.039$; $P = 0.018$ concentric; isometric and eccentric performance respectively (Table 3.1). Ratings of perceived soreness following stepping exercise for the Ecc condition were higher than pre-exercise values at both time points (24 and 48 hour) ($P < 0.001$). For both measures of perceived soreness (stepping up and down) an interaction of treatment × time ($P < 0.001$) was observed (Table 3.1).

	Concentric Leg			***Eccentric Leg***		
	Pre	**24hr**	**48hr**	**Pre**	**24hr**	**48hr**
Isometric (Nm)	229(8)	223(12)	224(8)	217(16)	174(23)*†	178(25)
Concentric (Nm)	195(11)	188(11)	188(11)	186(15)	146(16)*†	137(19)*†
Eccentric (Nm)	245(24)	257(14)	243(12)	238(19)	193(23)*†	183(25)*†
VAS stepping up (cm)	0(0)	0.6(1.2)	1(2.1)	0(0)	4.9(2.0)*†	7(2.3)*†
VAS stepping down (cm)	0(0)	0.6(0.7)	1(1.4)	0(0)	5.4(2.3)*†	6.8(2.4)*†

Table 3.1. Measures of knee extensor muscular strength and perceived muscle soreness following strenuous eccentric exercise (n = 8). (VAS – visual analogue scale). Data are means (SD). *Indicates significantly different from concentric leg at same time point. †Indicates significantly different from pre-measure ($P < 0.05$).

Creatine kinase

Capillary blood CK activity increased over time ($P < 0.001$) with all post-exercise values elevated above baseline levels (Figure 3.3). The highest CK value recorded occurred 48 hours after the box-stepping protocol at 468 $IU.L^{-1}$ (range 80 – 1510 $IU.L^{-1}$).

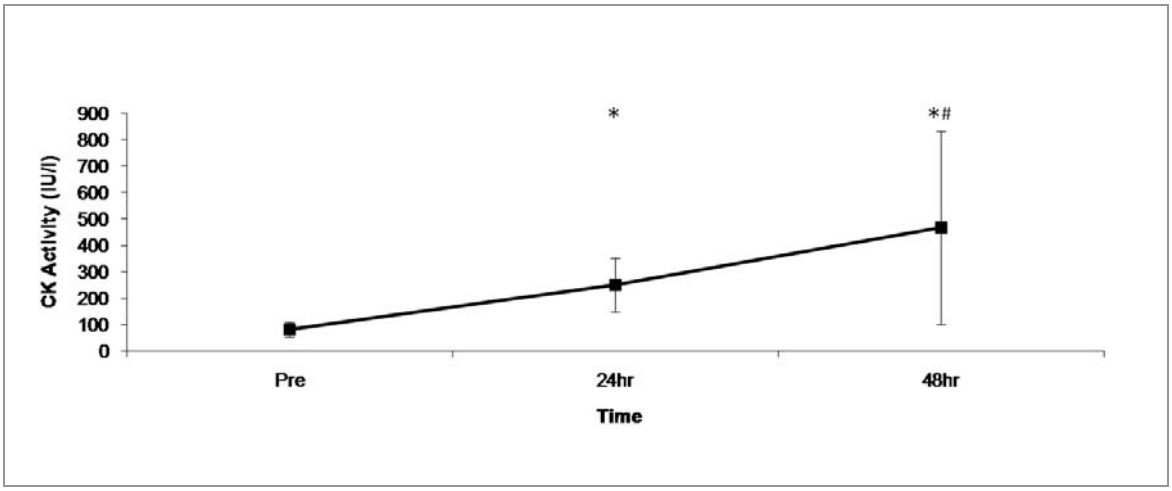

Figure 3.3. CK activity following Bench Stepping Exercise (BSE) (n = 8). Data are means (SD). *Indicates significantly different from pre. † Indicates significantly different from 24 hour measure ($P < 0.05$).

One legged cycling exercise

Both treatment × time interaction ($P = 0.017$) and treatment × time × effort ($P = 0.010$) interactions were observed for RER. The effect of the Ecc (treatment) on subsequent exercising rate of substrate utilisation was also revealed as a significant treatment × time interaction ($P = 0.021$) for calculated rate of CHO oxidation. Further, a significant treatment × time × workload ($P = 0.005$), indicated that the effect of the treatment altered as workload increased. Accordingly, the rate of fat utilisation revealed there were treatment × time interaction ($P = 0.034$) and treatment × time × workload, ($P = 0.030$) interactions for the rates of lipid oxidation during cycling exercise.

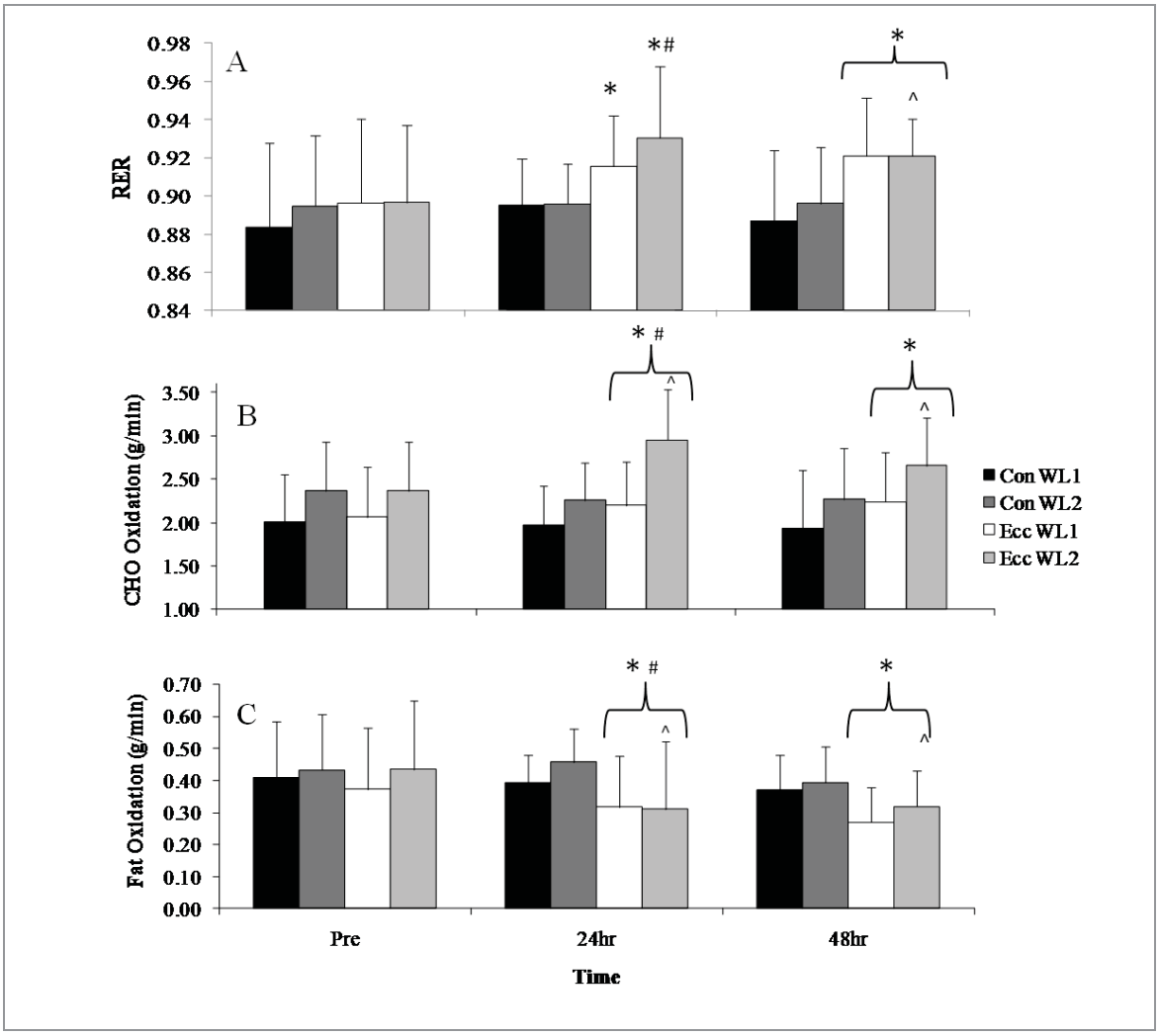

Figure 3.4. Respiratory exchange ratio (A), CHO oxidation (B) and fat oxidation (C) in Con and Ecc legs following BSE (n = 8). Data are means (SD). * Indicates significantly different from concentric trial (p<0.05). # Indicates significantly different from pre-measure ($P < 0.05$). ^ Indicates significantly different from prior workload ($P < 0.05$).

A main effect of effort ($P < 0.001$) was observed in the blood lactate response to the cycling exercise, but no significant effects of the treatment (i.e. differences between Ecc and Con) were apparent. An interaction of treatment × time was observed ($P = 0.006$) indicating that there was an increase in HR with the Ecc treatment across the time course of the experimental protocol. An interaction of treatment × time ($P = 0.004$) was observed, indicating that participants found it harder to cycle following Ecc than Con following BSE.

3.4 Discussion

The main purpose of this study was to investigate whether EEIMD could induce measurable changes in whole body substrate utilisation during subsequent concentric exercise.

Completion of the BSE resulted in significant decreases in muscular performance measures of the eccentric exercised leg only (Table 3.1). These losses in maximal voluntary tension in the eccentrically exercised leg were coincident with significant increases in measures of perceived soreness and blood CK activity. The decrement in force generation, combined with increased perception of pain and elevated CK activity are manifestations of EEIMD that has been widely documented using similar protocols (Newham et al., 1983a, Vissing et al., 2008). This demonstrates that the BSE resulted in exercise induced damage in one leg (Ecc), but not in the other (Con), despite matching external work done.

Because circulating substrate availability would have been the same when cycling with either leg, the observed increased rate of CHO utilisation in Ecc suggests that damaged muscle has an increased reliance upon glycogen oxidation. It is possible that EEIMD alters the rate of contraction induced glucose uptake, either increasing the reliance upon glycogen by inhibiting glucose uptake, or increasing the capacity to take up and oxidise glucose rather than lipids and thereby producing a higher RER. Research measuring arterio-venous difference is required to elucidate this further.

It has been shown that IMCL will accumulate in situations where muscle glycogen cannot (Zderic et al., 2004), so this suggests a relatively impaired ability to accrue glycogen following damage. In support of this are the observations of others that glucose uptake and glycogen resynthesis is impaired following EEIMD (Costill et al., 1990, Widrick et al., 1992, Asp et al., 1998, Asp et al., 1995). However, the results of the present study seem to conflict with these findings. Usually, higher rates of an intramuscular substrate are strongly associated with its preferential utilisation during exercise (Boesch et al., 1997, Gollnick et al., 1972, Hargreaves et al., 1995, Rico-Sanz et al., 2000, Schrauwen-Hinderling et al., 2003, White et al., 2003), so it would be

expected that there would be a reduced rate of CHO oxidation in Ecc. However in the present study the opposite was observed.

EEIMD results in increases in skeletal muscle protein degradation and synthesis. However, the likely energy cost of these processes is difficult to fully ascertain, though they may contribute to an increase in metabolism following EEMID. Recently it has been hypothesised that EEIMD causes alterations in homeostasis through the requirement of energy to repair muscle tissue (Dolezal et al., 2000). This has been confirmed in number of studies (Chesley et al., 1992, Fielding et al., 1991) where maximum rates of muscle protein turnover lasted for 2 days in response to an acute bout of heavy eccentric resistance exercise. Although the present study did not measure protein turnover, it could be speculated that the stepping protocol influenced myofibrillar protein metabolism for a period of time post exercise. Because the energy cost of protein turnover may account for as much as 20% of resting metabolism (Welle and Nair, 1990), the energy utilisation during the 48 hours recovery period for the present study may have caused elevations in the metabolic rate during the subsequent bout of exercise.

Changes in efficiency during the cycling exercise as a function of Ecc could explain our results, although this was not seen between pre- and post BSE. Others have shown that during strenuous cycling exercise post-eccentric exercise O_2 kinetics are preserved (Schneider et al., 2007) probably through an increase in localised muscle blood flow (Davies et al., 2008), which compensates for microvasculature dysfunction that occurs following eccentric exercise (Kano et al., 2004).

Participants in the present study reported higher ratings of perceived exertion (RPE) during the cycling exercise when using the eccentrically exercised leg. It has been reported that during cycling exercise RPE are based on a combination of muscular pain and the sensation of breathlessness (Jameson and Ring, 2000). Therefore the muscular pain following the eccentric exercise provides a peripheral cue to the RPE while the ventilatory response informs us of a central cue. A more recent study utilising a single leg design showed that there were no changes to cycling power or RPE in the concentrically exercised leg whilst changes to RPE and cycling power in the

eccentrically exercised leg gave evidence that peripheral muscle damage would be the main instigator of a higher RPE when cycling (Elmer et al., 2010).

In conclusion, the performance of eccentric exercise resulted in decrements in muscle strength 24 and 48 hours after exercise, which is indicative of damage to muscle fibers. This decline in strength after eccentric exercise was associated with a higher reliance upon intramuscular CHO oxidation during concentric work.

4. Effects of eccentric exercise induced muscle damage on intramyocellular lipid concentration and high energy phosphates

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

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.*

4.1 Abstract

Eccentric exercise is known to cause changes to the ultrastructure of skeletal muscle and, in turn, may alter the ability of the muscle to store and utilise intracellular substrates such as intramyocellular lipid (IMCL). The purpose of this study was to test the hypothesis that EEIMD results in IMCL accumulation. Six males (mean ± SD; 31 ± 6 yrs and 72.3 ± 9.7 kg body mass) performed 300 unilateral, maximal, isokinetic, eccentric contractions (Ecc) ($30°.s^{-1}$) of the quadriceps of one leg on an isokinetic dynamometer, followed immediately by an equal amount of work by the contralateral leg but with concentric action (Con). Phosphate compounds and IMCL content of the *vastus lateralis* of both legs were measured using ^{31}P-MRS and ^{1}H-MRS, respectively. IMCL content was higher in Ecc than Con 24 hours post exercise but the reverse was evident 48 hours post exercise ($P = 0.046$). A significant time × treatment interaction was evident for resting [P_i] ($P = 0.045$), which increased with time in Ecc but not in Con. Further, a significant main effect for treatment ($P = 0.002$) for resting [P_i] was apparent, which is consistent with metabolic alteration in the Ecc leg. The P_i / PCr ratio showed a significant main effect for treatment ($P = 0.001$) with an increase evident in Ecc leg, primarily due to the increase in [P_i]. The present study shows that EEIMD is associated with alteration of the IMCL pool in skeletal muscle and increases in [P_i].

4.2 Introduction

Although muscle contraction improves sensitivity of skeletal muscle to insulin-mediated glucose uptake in the short and longer term (Holloszy et al., 1998), paradoxically, strenuous eccentric muscular work has been shown to reduce resting muscle insulin sensitivity (del Aguila et al., 2000). Although there is no definitive explanation for this impairment, it has been proposed that the reduced insulin sensitivity results from a systemic inflammatory response to EEIMD (Costill et al., 1990).

Whole body insulin resistance in obesity, lipid infusion and starvation is closely associated with stagnancy and subsequent accumulation of the triglyceride pool and other lipid compounds in skeletal muscle (Kelley et al., 2002, Stannard and Johnson, 2006). Accumulation of this IMCL occurs when the rate of fatty acid uptake by the muscle fibre exceeds the rate of fatty acid and IMCL-derived fatty acid oxidation in the mitochondria. Thus, any situation where the opportunity to oxidise lipid is reduced, (such as inactivity or mitochondrial damage) and/or where lipid availability is increased, (such as in starvation, lipid/heparin infusion and obesity), will result in lipid accumulation and reduced insulin sensitivity.

Ultrastructural changes of the mitochondria skeletal muscle have been identified following eccentric exercise (Su et al., 2010) so that EEIMD may provide conditions conducive to reduced lipid oxidation, IMCL accumulation and reduced insulin action. Furthermore, it has been suggested that eccentric-based exercise might increase the uptake and storage of blood lipid and lipoprotein-derived fatty acids within muscle as evidenced by decreases to circulating triacylglycerols (TG), total cholesterol (TC), and increases to high-density lipoprotein cholesterol (HDLC) (Nikolaidis et al., 2008). Yet, to date, the effect of EEIMD on IMCL concentration has not been measured.

Increases in [P_i] have been shown to occur following EEIMD (McCully et al., 1988). A significant increase is also evident in the resting P_i / PCr ratio following eccentric exercise (Aldridge et al., 1986, Lund et al., 1998b, McCully et al., 1988). This could be interpreted as an increase in cell metabolism (i.e. ATP consumption) following the muscle injury and there is some evidence that disruption to the sarcolemma and the sarcoplasmic reticulum can cause an increase in ion-pumping activity and protein

synthesis following EEIMD (Byrd, 1992, McNeil and Khakee, 1992). Both of these could lead to an increase in resting metabolism. However, sarcolemma disruption can increase the [P_i] without alteration in [PCr] (Aldridge et al., 1986) thereby increasing P_i / PCr ratio, consistent with a mobile pool of P_i. This change in P_i / PCr is therefore employed as an unspecific marker of muscle damage (Lund et al., 1998b, Lund et al., 1998a, Rodenburg et al., 1994).

The purpose of this study was to test whether EEIMD alters intracellular lipid concentration. Six men performed 300 eccentric contractions of one leg and then completed the equivalent work, concentrically, with the other leg. At 24 and 48 hours after this exercise, resting ^{1}H-MRS and ^{31}P-MRS metabolite concentrations were measured at rest in each leg. An improved understanding of the role IMCL plays in skeletal muscle recovery from EEIMD may help to further enhance our knowledge of skeletal muscle lipid metabolism.

4.3 Methodology

Participants

Six males (mean ± SD; age 31 ± 6 yr, height 176.4 ± 6.8 cm and body mass 72.3 ± 9.7 kg) who had not been exposed to eccentrically biased exercise in the six months preceding the study volunteered to participate in this research. None of the participants had a history of muscle or metabolic disease. All participants completed a medical screening questionnaire and provided written informed consent. Participants were asked to refrain from strenuous exercise for the preceding two weeks and for the duration of the study intervention. The study was performed according to the Declaration of Helsinki and approved by the University's Research Ethics Committee prior to the start of the investigation.

Experimental overview

Participants attended the laboratory to complete a bout of maximal voluntary exercise. This involved 300 maximal voluntary eccentric contractions across the knee of one leg then, after 10 minutes rest, maximum voluntary contractions on the other leg until the same total work was completed. Legs were randomly assigned to induce damage in the dominant or non-dominant leg, but in a balanced fashion so that half the subjects completed the eccentric exercise with their dominant leg and half with their non-dominant leg. Exactly 24 and 48 hours after this exercise, subjects underwent both ^{31}P-MRS assessment of the *quadriceps* which incorporated the *vastus lateralis* and ^{1}H-MRS assessment of the *vastus lateralis* of both legs. The 24 and 48 hour time points were chosen because these coincide with the greatest change in the ultra-structure of the muscle fibre and pronounced feelings of muscle soreness (Clarkson et al., 1992) and rather than look at pre- post differences it was of greater importance to capture the temporal alterations following eccentric exercise. It has also been established that glycogen resynthesis is inhibited during the initial 48 hour period post eccentric exercise (Costill et al., 1990, O' Reilly et al., 1987) and this may influence IMCL metabolism (Kelley et al., 2002, Stannard and Johnson, 2006).

This novel concurrent single leg design allows us to control for systemic factors such as diet and circulating cytokines (Smith et al., 2000), which may influence IMCL accumulation (Johnson et al., 2006, Kiens and Richter, 1998).

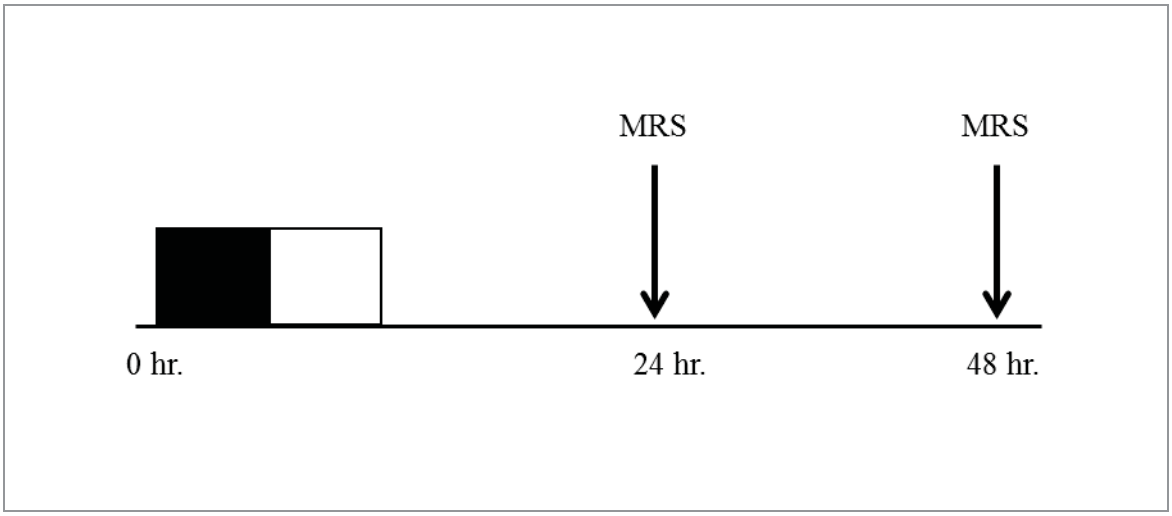

Figure 4.1. Schematic presentation of the experimental protocol. Indicating, eccentric exercise (black box) and concentric exercise (white box), measures of ^{31}P & ^{1}H-MRS (MRS) that were conducted post eccentric and concentric exercise 24 and 48 hour post.

Eccentric exercise protocol

The isokinetic dynamometer (Biodex Medical Systems, New York, USA) was set up as recommended by the manufacturer to exercise the knee extensors, including the *vastus lateralis*. The dynamometer was set to an angular velocity of $30°.s^{-1}$ in the passive mode. At maximal knee flexion (0°) whilst seated on the dynamometer, participants were instructed to extend their knee through a ROM of 60° (1.05 rad); this was to be the ROM for the exercise protocol. This protocol has previously been shown to bring about significant levels of muscle damage and soreness (MacIntyre et al., 1996). The ROM was measured using a protractor goniometer; the stationary arm of the goniometer was centred on the axillary line of the femur and oriented with the lateral femoral epicondyle. The moving arm was in line with the longitudinal axis of the lower leg, oriented on the tip of the fibular head and the middle of the inferior side of the lateral malleolus. During the eccentric exercise, the participants were instructed to resist maximally through the entire 60° ROM and then completely relax through the passive extension phase. Eccentric exercise performed at long muscle lengths results in greater

functional impairment and evidence of muscle damage than eccentric exercise performed at short muscle lengths (Byrne et al., 2001). Total work done (Joules) was recorded (LabView, Eagle Scientific, USA) and this work provided an endpoint for the concentric exercise. The concentric exercise was conducted with identical subject specific ROM. The ROMs were selected to exercise the knee extensors at long muscle length.

The participants performed 300 unilateral, maximal, isokinetic, contractions ($30°.s^{-1}$) of the quadriceps, split into three sets of 100 maximal eccentric contractions with no rest between contractions but a 5 minute rest between sets. Then, after 10 minutes rest, this was followed by an equal amount of work done by the knee extensors of the contralateral leg with concentric contractions. Visual feedback from the dynamometer and standardised strong verbal encouragement from the investigator were given during the eccentric exercise to encourage maximal effort throughout the protocol and to ensure tension was applied through the full ROM. Subjects were asked not to participate in strenuous exercise for the two weeks preceding their participation and for the duration of the protocol.

Magnetic resonance spectroscopy

The MRS consisted of four separate measures to determine the IMCL content and intracellular phosphate concentrations in both legs. Participants were first assessed for IMCL content of the *vastus lateralis* by localised ^{1}H-MRS. Participants were then scanned using ^{31}P-MRS to derive resting muscle [PCr], [TCr] and [P_i] of the *quadriceps* muscle (incorporating the *vastus lateralis)* of the same leg. These measures were made for the purpose of confirming EEIMD (McCully et al., 1992), because we cannot confirm this with circulating markers (e.g. CK) using the study design employed. All measurements were repeated on the contra-lateral leg.

For the purpose of this study the Cr signal from the ^{1}H-MRS spectra was utilised as the internal standard and hence the IMCL signal was referenced to Cr. The Cr peak was referenced rather than the water peak because EEIMD is known to result in oedema (Rodenburg et al., 1994). The contralateral leg served as a control in all tests before and after exercise. No dietary restraints were placed on the participants though a record of what was eaten over the course of the experiment was taken to ensure a eucaloric diet

was maintained. No exercise apart from walking was permitted to be undertaken in the 48 hours after the eccentric exercise.

All MRS measurements were acquired using a 1.5 Tesla Intera whole-body system (Philips Medical Systems, Best, Netherlands). For determination of IMCL concentration a 5.0 cm x 1.5 cm x 1.5 cm voxel was centred within the *vastus lateralis* at the level of mid-femur according to the method outlined previously (Johnson et al., 2006). Briefly, legs were fixed with the thigh orientated so that the muscle fibres of the *vastus lateralis* were aligned with the main magnetic field (B_0). Localised spectra were acquired using the point resolved spectroscopy (PRESS) technique (TR = 5000 ms, TE = 32 ms, 64 measurements, 1024 sample points). Fully automated high-order shimming was performed on the volume of interest to ensure maximum field homogeneity. Excitation water suppression was used to suppress the water signal during data acquisition.

During MRI imaging and the ^{31}P-MRS scan, participants lay prone with one leg restrained by straps, which held the quadriceps firmly against a P-10 (10 cm diameter) pulse-receive coil (Philips Medical Systems, Best, Netherlands) positioned at mid-thigh (Figure 4.2). For determination of muscle volume, axial dual-echo images were also acquired using the Q body coil (slice thickness 6 mm; slice gap 30 mm) from below the knee joint to above the hip joint. Using the previously acquired images, a volume of interest was chosen to shim the magnetic field to muscle water using the Q body coil. The phosphorus coil was then manually matched (50 Ω) and tuned to the phosphorus frequency (25.85 MHz). ^{31}P free-induction decay (FID) spectra were acquired (TR = 15000 ms; 8 measurements) allowing full T1 (longitudinal) relaxation. The spectra were non-localised, acquired using an adiabatic pulse with proton decoupling and nuclear overhauser enhancement (NOE).

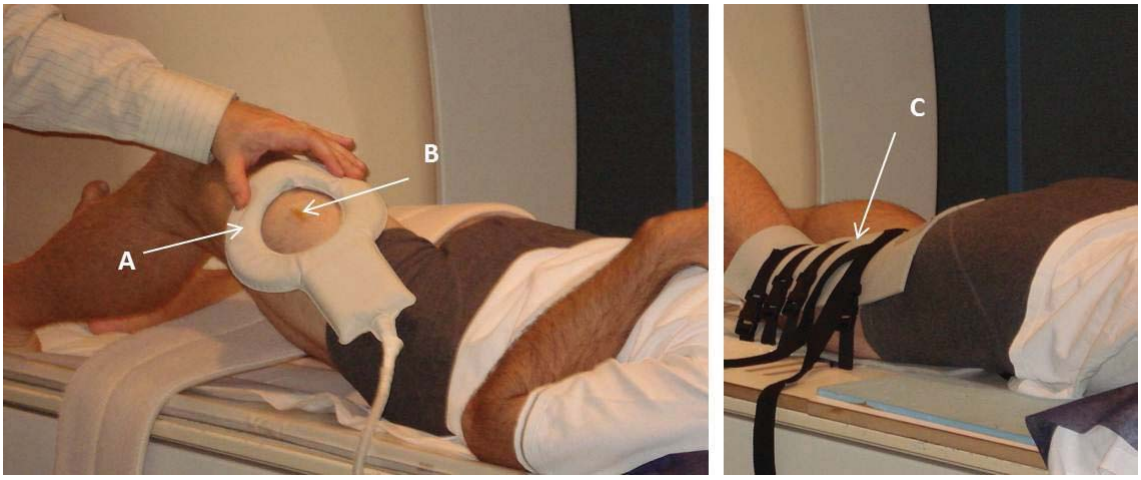

Figure 4.2. Positioning of the P-10 receiver coil and leg positioning for MRS measurements. (A) P-10 receiver coil, (B) vitamin E capsule for marking site of interest for voxel placement, (C) straps positioned over thigh.

Spectral analyses and muscle volumes

Commercially available software (ImageJ, NIH, USA, http://rsb.info.nih.gov/ij/) was used to manually outline the *quadriceps* (*rectus femoris, vastus lateralis, vastus intermedius* and *vastus medialis*) muscles on the selected slices (Figure 4.3). The total volume of each muscle (cubic centimetres) was calculated by summing the product of the measured muscle cross sectional areas (CSAs) and the slice thickness for all slices in each participants legs and the volume of the gaps between the slices was estimated with the truncated cone formula (Ross et al., 1996). All volume analysis was done by a single investigator (the first author) to ensure consistency across subjects and time.

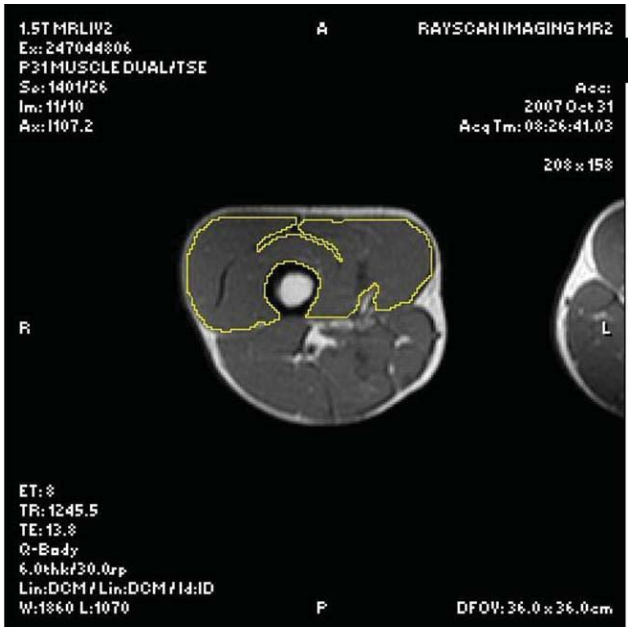

Figure 4.3. Representative mid-quadriceps axial plane MRI from 1 subject after eccentric exercise bout. The outlined CSA are used in the calculation for muscle volume as described above.

For determination of IMCL content a ten resonance model was used, which included IMCL (-CH_3: 0.88ppm), EMCL (-CH_3: 1.19ppm), IMCL (-$(CH_2)_n$: 1.3 ppm), EMCL (-$(CH_2)_n$: 1.51 ppm, L-acetate (2.09 ppm), lipid (C_2 methylene: 2.24 ppm), total creatine (-CH_3: 3.02 ppm), TMA (Trimethylacetate group) (3.2 ppm), taurine (-N- CH_3: 3.37 ppm and residual water signal (4.69 ppm). The (total) creatine peak (Cr) was identified and nominated 3.02 ppm and used as the internal standard. The spectra were processed using a JAVA-based magnetic resonance user interface (jMRUI version 3.0, EU Project). Excitation water suppression was used to suppress the water signal during data acquisition. Resonance amplitudes were obtained via advanced method for accurate, robust and efficient spectral fitting (AMARES) using prior knowledge described previously (Johnson et al., 2003b).

^{31}P-MRS spectra were also analysed for the relative amplitudes of PME, PCr, P_i and α-, β- and γ-ATP. All signals were fitted without any pre-processing or phase correction. T1 saturation effects were corrected relative to the initial resting spectra. Amplitudes

were corrected for surface coil offset, proton decoupling and Nuclear Overhauser Effect (NOE). The [PCr] and [P_i] were then calculated relative to β-ATP, assuming a constant [ATP] of 8.2 mmol L^{-1} (Taylor et al., 1986). The chemical shift between PCr and P_i was used to measure muscle pH (Moon and Richards, 1973). ^{31}P-MRS derived total basal muscle creatine (TCr_{BASAL}) was calculated assuming PCr = 0.61 x Cr (Rico-Sanz et al., 1998). The concentration of free adenosine diphosphate [ADP] was calculated as:

$$[ADP] = [\sum ATP](([\sum TCr]/[\sum PCr]) - 1)/(K_{CKapp}[H^+])$$

where K_{CKapp} is a function of the binding of H^+, K^+ and Mg^{2+}, which in turn is a function of pH (Kemp et al., 2001).

Statistical analyses

All statistical analyses were performed using PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL, USA). All data are reported as the mean ± SD. *V.lateralis* IMCL content and resting ^{31}P metabolite concentrations were compared via a two-way repeated-measure ANOVA (time × treatment). Muscle volume measurements were compared using paired two-tailed *t*-tests at 24 then 48 hours separately, rather than a two-way ANOVA. This was because the image from one participant at 48 hours was corrupted and unable to be analysed. Statistical significance was accepted at $P < 0.05$. The small sample sizes in this study were unavoidable because of the high cost of the magnet time. Further, there was a dearth of relevant prior published work from which an Effect Size could be calculated.

4.4 Results

All subjects completed 300 eccentric contractions (Ecc) in one leg and an average of 418 ± 47 concentric contractions (Con) using the contra-lateral leg to ensure total external work done was equivalent between legs.

All subjects reported extreme delayed soreness in the eccentrically exercised leg, compared with the concentrically exercised leg, peaking between 48 and 72 hours. This therefore, provides supporting evidence that the exercise protocol was sufficient to engender significant muscle damage.

Skeletal muscle ^{1}H-MRS.

No significant differences were found in the total creatine signal of either the Ecc or Con leg throughout the duration of the treatment ($P = 0.687$). A main effect of time was evident ($P = 0.046$) with an increase in IMCL/Cr in Con from 24 to 48hours. IMCL/Cr signal showed a significant time × leg interaction whereby there was an increase in IMCL/Cr with time in Con relative to Ecc ($P = 0.032$) (Figure 4.4). The interaction between conditions and time; was such that IMCL was higher at 24 hours in Ecc versus Con, with no further increases in content after that. In contrast, the IMCL content of Con was lower at 24 hours and increased over the subsequent 24 hours.

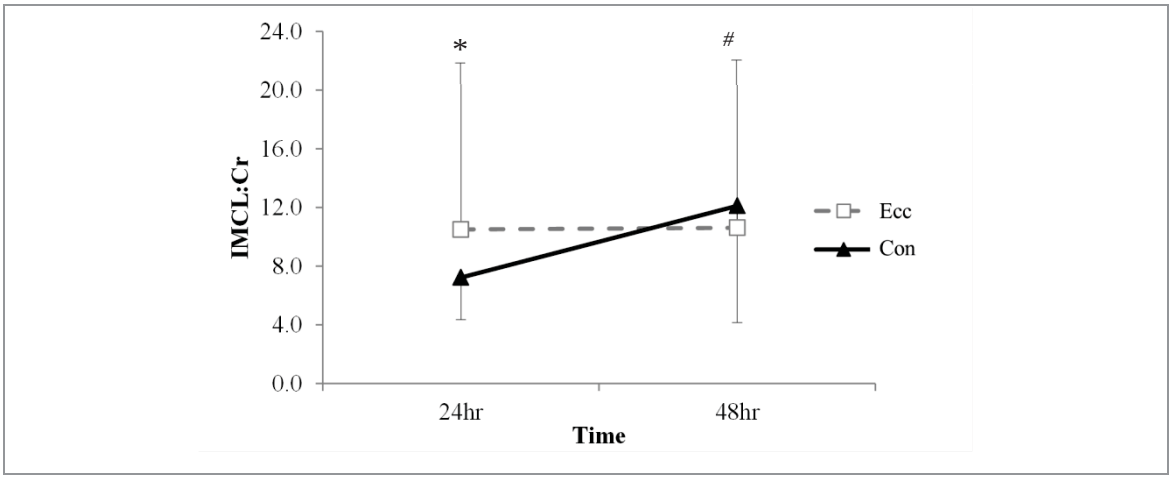

Figure 4.4. IMCL: Cr in both experimental (eccentric) and control (concentric) legs at 24 and 48 hours post exercise (n = 6). * Indicates significantly different from control. #Indicates significantly different from control 24 hour measure.

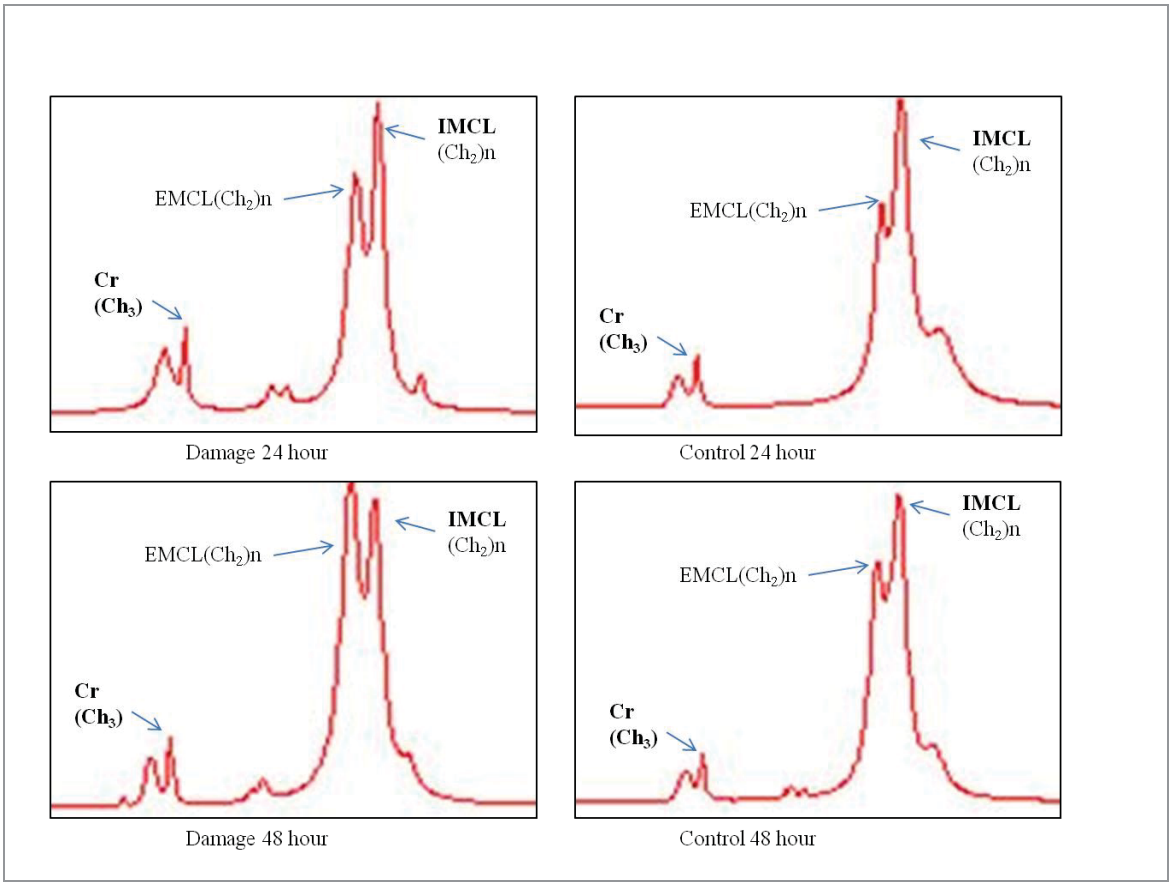

Figure 4.5. Representative ^{1}H-MRS spectra for both conditions (Ecc and Con) at both time points (24 and 48 hours) of human skeletal muscle at 1.5Tesla showing the lipid peaks of IMCL and EMCL and Cr peak after water suppression. Spectra are taken from the same participant.

Skeletal Muscle 31P-MRS – Resting measures

There were no significant main effects for treatment or time for resting [PCr] (P = 0.170, P = 0.735 respectively) between legs. There was a significant time × treatment interaction for resting [P_i] (P = 0.045) and significant main effect of treatment (P = 0.002), such that P_i increased in Ecc over time but not in Con. There was a significant effect for treatment on resting P_i / PCr ratio (P = 0.001) with an increase in the ratio being evident in the damaged leg, primarily due to the increase in P_i. There were no significant main effects for treatment or time on resting [ADP] (P = 0.963, P = 0.294 respectively). There were no significant main effects of treatment or time between legs for resting pH (P = 1.000, P = 0.415 respectively).

	Control24hr	*Control 48hr*	*Damage 24hr*	*Damage 48hr*
Muscle volume (cm^3)	1121 (203)	1117 (209)	1254 (233) *	1197 (236)
[PCr] ***(mM)***	26.66 (4.72)	23.50 (4.23)	25.45 (4.80)	28.91 (3.76)
[P_i] ***(mM)***	4.06 (0.74)	3.58 (1.11)	5.41 (1.48)*	6.10 (1.18)*#
P_i /*PCr*	0.15 (0.02)	0.15 (0.04)	0.21 (0.03)*	0.21 (0.03)*
[ADP] (µM)	43.4 (1.67)	43.74 (1.69)	43.25 (3.80)	44.06 (3.00)
pH	7.01 (0.02)	7.02 (0.02)	7.01 (0.04)	7.02 (0.03)

Table 4.1. *Quadriceps* resting muscle volumes and metabolite concentrations in *V. lateralis* of control and damaged legs (n = 6). Data are means (SD). *Indicates significantly different from control. #Indicates significantly different from 24 hour measure ($P < 0.05$).

Quadriceps muscle volume

Measured muscle volume was significantly larger in the eccentrically exercised leg at 24 hours compared to the control leg ($P = 0.014$). There was no significant difference between conditions at 48 hours ($P = 0.118$).

4.5 Discussion

The aim was to compare the metabolic consequences of equivalent concentric and eccentric work. Changes to mitochondria ultrastructure following eccentric exercise (Su et al., 2010) may be conducive to reduced lipid oxidation and IMCL accumulation. Favourable changes to the lipid and lipoprotein profile following eccentric exercise might increase the uptake and storage of fatty acids from circulating FFAs and lipoproteins within muscle (Nikolaidis et al., 2008). A higher [IMCL] in *v.lateralis* was observed 24 hours post eccentric exercise. The advantage of the one-legged model employed over a cross-over design where the same leg is measured on two different occasions (such as walking up, then separately down a hill) is that the same circulating substrates, hormones and cytokines were available to both legs concurrently in the recovery period. This is especially important because the availability of circulating substrates has a primary influence on IMCL repletion after exercise (Johnson et al., 2006, Kiens and Richter, 1998) and any necessary walking during recovery similarly incurs the same metabolic cost in each leg. Therefore, any observed differences between legs in the dependent variables measured must result from changes within the muscle itself.

Also observed was a metabolic alteration within the *m. quadriceps femoris* that had undertaken the eccentric protocol, which was reflected in the higher [P_i] observed 24 and 48 hours post exercise. Elevated [P_i], a product of PCr hydrolysis, is associated with fatigue in healthy muscle and dysfunction in diseased muscle. The ratio of P_i /PCr has been utilised to reflect the degree of metabolic activity that is occurring within skeletal muscle (McCully et al., 1988). Alterations in this ratio can occur in a number of circumstances, such as muscular myopathies, metabolic disorders and post exercise fatigue from both short and long duration exercise protocols (Baker et al., 1993, Widrick, 2002). The data from this study showed the P_i / PCr ratio measured in the control condition as 0.15 ± 0.04, which is in agreement with values reported in the literature (Vandenborne et al., 1995). The data from this study revealed both an increase in resting [P_i] and an increase in the P_i / PCr (0.21 ± 0.03) in the eccentrically exercised leg. Both these non-specific markers of muscle damage can be considered to be evident in the eccentrically exercised leg and absent from the concentrically exercised leg.

This is the first study to show that skeletal muscle exposed to very strenuous eccentric exercise and therefore EEIMD, responds differently in terms of IMCL accumulation in first 48 hours of recovery. Previous reports have observed a reduced ability to accrue muscle glycogen following EEIMD (Widrick et al., 1992) and that IMCL accumulates when glycogen is unable to accrue (Stannard and Johnson, 2004). It was therefore expected that a relative increase in IMCL in the treatment (damaged) leg would occur. Instead, a significant interaction between conditions and time was observed: the damaged leg showing higher IMCL at 24 hours but did not increase in content after that. In contrast, the IMCL content of the previously concentrically contracted leg was lower at 24 hours and increased in content over the subsequent 24 hours period.

This observation could be interpreted in a number of ways. Firstly, whilst the external work was balanced between legs, the increased negative work borne by the passive (non-contractile) elements in the muscle during eccentric work (Fridén et al., 1983b), means that the metabolic cost of contraction was higher in the control (concentric) leg. This, along with the fact that greater number contractions were performed in the control leg, means that more IMCL may have been used as a fuel during the concentric contractions. The lower concentrations of IMCL seen at the 24 hour period may therefore reflect partial recovery of that substrate store. In support of this hypothesis, eccentric exercise appears not to follow the 'Size Principle' (Nardone et al., 1989), such that there may be relatively greater reliance upon the larger, less oxidative motor units during strenuous negative work. The treatment (eccentric) leg may therefore have used proportionally more glycogen to support its 300 contractions, with less reliance upon the more oxidative (smaller) motor units. A limitation of this study is that measures of [IMCL] were not collected prior to the exercise interventions.

A second explanation for the observed changes in IMCL may involve changes in resting creatine resulting from EEIMD. As stated in the methodology, the Cr signal from the ^{1}H-MRS spectra was utilised as the internal standard and hence the IMCL signal is referenced to Cr. The IMCL peak was referenced to the creatine peak rather than the water peak because EEIMD is known to result in oedema. However it cannot be excluded based on the data gathered that the water retention may have affected the T1 and T2 (transverse) relaxation times differently. Significantly larger changes in muscle volume in the treatment leg (Table 1) were observed, which is consistent with this.

Thus, if the intracellular creatine concentration is altered by EEIMD, then this may affect the measure of apparent [IMCL]. However, Hesselink *et al.* (1998) showed that the PCr content of rat *tibialis anterior* subjected to 240 eccentric contractions and resulting in the structural changes associated with sustained eccentric work, was not different after 24 hours recovery in comparison to the contralateral control muscle (Hesselink et al., 1998). The proportion of PCr that constitutes the total creatine (TCr) pool is assumed to be stable in resting skeletal muscle and has routinely been measured using ^{31}P-MRS to indicate [TCr] (Kemp et al., 2007). The data from this study similarly reveal that there was no difference in [PCr] between the two conditions and it can therefore be assumed that the usage of the Cr signal as an internal reference to quantify IMCL is valid, though this may warrant further investigation.

Nikolaidis et al (2008) observed decreases in the blood lipid profile post eccentric exercise. They speculated that these may have been brought about by the increase in activity of lipoprotein lipase (LPL) that promotes lipolysis and FFA release, which in turn, could be taken up by the skeletal muscle and then either oxidised or esterified as IMCL. However, the current study can go some way to disproving this speculation. As both the control and damaged muscle were exposed to the same circulation (and thus lipoprotein/FFA delivery), any differences observed between legs in IMCL content must be due to intramuscular factors. However, it has been established that unaccustomed eccentric exercise can lead to substantial microvascular dysfunction (Kano et al., 2004). There is contradictory evidence as to whether this microvascular dysfunction affects muscle oxygenation, seeing as measures of muscle oxygenation are concerned with the transition from rest to intense exercise. It is therefore hard to establish whether the changes exist in a rested state and would therefore impact on the results herein.

The present study has highlighted that strenuous eccentric exercise resulting in EEIMD produces changes in IMCL content and that this is associated with altered [P_i] in the muscle. Since EEIMD is also associated with impaired insulin-mediated glucose uptake, our observation may help to shed further light upon the role of skeletal muscle lipid metabolism in the development of EEIMD induced insulin resistance. Further, it is evident that high energy phosphate compounds are not changed at rest in muscle exposed to prior eccentric contractions and therefore considered to be damaged.

5. Repeat bout effect on eccentric exercise induced muscle damage and glycaemic response

5.1 Abstract

The purpose of this study was to investigate whether disruptions to glucose and insulin responses to an OGTT following eccentric exercise can be attenuated after a repeated bout of eccentric exercise. Eight male participants (mean ± SD, 31 ± 7 yr, 177.7 ± 4.6 cm, 78.9 ± 9.7 kg and 17.4 ± 2.7% body fat) performed an initial bout of squat exercises (SE1), followed 14 days later by a repeat bout of squat exercises (SE2). OGTTs were administered at baseline and 48 hours after SE1 and SE2 and measures of circulating glucose and insulin were taken. Fasting levels of glucose, insulin, serum CK activity and performance measures (MVC, counter jump performance (CMJ) and perceived soreness) were assessed pre, 24, 48 and 96 hours post SE1 and SE2. A significant interaction of trial × time was evident for CK activity such that the activity increased from the pre measure, with biggest greater increase in SE1 ($P = 0.006$). A significant interaction of trial × time for CMJ ($P = 0.022$) was shown with a decrease in height following SE1. A main effect of trial for perceived soreness ($P < 0.001$) was revealed between SE1 and SE2 indicating a repeat bout effect had occurred. There were no significant differences in the fasting insulin ($P = 0.968$) and glucose levels ($P = 0.834$) following either SE1 or SE2. There were also no significant differences in the response of glucose or insulin ($P = 0.905$; $P = 0.762$ respectively) to the OGTT following the SE1 and SE2 in comparison to a control trial. The area under the curve (AUC) for both glucose and insulin following SE1 and SE2 also showed no significant difference from that of the control measure ($P = 0.742$ and $P = 0.955$). In conclusion, a bout of eccentric biased exercise resulted in mild muscle damage. Recovery from the initial bout of eccentric exercise conferred a protective effect on the muscles performance following a second bout of the same eccentric exercise performed 14 days later. However, this study did not reveal an elevation in the insulin response after a novel bout of eccentric exercise and further that a subsequent bout of eccentric exercise attenuated the responses.

5.2 Introduction

Alterations to the normal physiological response to increases in blood insulin concentrations, defined as 'transient insulin resistance' (Kirwan and del Aguila, 2003), have been shown to occur following eccentrically biased exercise (Asp et al., 1996, King et al., 1993, Kirwan et al., 1991, Kirwan et al., 1992, Sherman et al., 1992). These alterations in whole body glycaemic control following eccentric exercise are associated with reductions in the quantity of glucose transporters available for translocation to the cell membrane (Asp et al., 1995) and therefore, reduce the muscles ability to adequately synthesise glycogen following eccentric biased exercise (Costill et al., 1990, Doyle et al., 1993, O' Reilly et al., 1987, Widrick et al., 1992). Skeletal muscle glucose metabolism is highly regulated by insulin and, under resting conditions, skeletal muscle accounts for approximately 85% of glucose disposal after glucose ingestion (DeFronzo et al., 1981, Yki-Jarvinen et al., 1987). Failure to adequately dispose of blood glucose after eccentric exercise could help explain the reduced rates of skeletal muscle glycogen synthesis.

Administration of an OGTT 48 hours following a bout of eccentric exercise revealed that peak insulin and insulin AUC responses were significantly higher than those reported for control conditions (Sherman et al., 1992), though the response to blood glucose was not altered. Likewise, administration of hyperglycaemic clamp 36 hour following eccentric exercise has shown that peak plasma insulin levels were matched with increases in insulin AUC (King et al., 1993). It was further demonstrated that insulin-mediated glucose disposal rates were impaired during a euglycemic-hyperinsulinemic clamp that was administered 48 hours following a 30 minutes downhill run (Kirwan et al., 1992). A plausible reason for this apparent impaired insulin-mediated glucose uptake is that eccentric exercise is known to cause damage to the membrane of muscle cells and it is this disruption of the membrane that affects the binding of insulin to its receptor (Ide et al., 1996).

Following an initial bout of eccentric biased exercise, skeletal muscle undertakes a repeat bout effect (RBE) (Byrnes et al., 1985). That is, a subsequent bout of identical eccentric work performed by the same muscle results in reduced muscle soreness,

reduced circulating CK activity and the ability to maintain force generating capacity by the muscle. This apparent protective effect of the initial bout of eccentric work on subsequent potentially damaging exercise has been evidenced to last for up to several weeks (Byrnes et al., 1985) or even months (Nosaka et al., 2001) and has been achieved with relatively few eccentric contractions (Clarkson and Tremblay, 1988, Nosaka et al., 2001).

Recent evidence indicates that the RBE can attenuate the insulin resistance, which occurs following an unaccustomed bout of eccentric exercise (Green et al., 2010). Green et al. (2010) utilised a 30 minutes downhill running protocol at 12% gradient to induce EEIMD. Participants subsequently ingested an oral bolus of glucose (1.6g/kg lean body mass) after an initial downhill run, then another after a second downhill run 14 days later. There was evidence for attenuation in the peak insulin response to an OGTT and an attenuation of the AUC for insulin after the second run. However, they also showed a glucose intolerance following the initial bout of eccentric exercise, something, which could be attributed to glucose dosage and to the participant characteristics.

The purpose of this study was to determine whether there would be alterations to the insulin and glucose response to an OGTT after a novel bout of eccentrically biased resistance exercise. Secondly, the responses to an OGTT would be studied following a second bout of eccentric contractions. The hypothesis was that the AUC for insulin in response to an OGTT would be attenuated after a second bout of squat exercise 2 weeks later and that the blood glucose response would not be different from baseline values after any of the trials.

5.3 Methodology

Participants

Eight males (mean ± SD age 31 ± 7 yr; 177.7 ± 4.6 cm; 78.9 ± 9.7 kg and 17.4 ± 2.7% body fat) who were considered concentrically trained but not accustomed to eccentrically biased exercise volunteered to participate in this study. None of the participants had a history of muscle or metabolic disease. All participants completed medical screening questionnaire and provided written informed consent. Participants were asked not to participate in strenuous exercise for the preceding two weeks and for the duration of the protocol. The study was performed according to the Declaration of Helsinki and approved by the University's Research Ethics Committee approved all procedures prior to the start of the investigation.

Experimental overview

Whole-body insulin action was indirectly examined through analysis of the glucose and insulin AUC resulting from an OGTT administered after a bout of novel eccentrically biased exercise (squat exercise). The same protocol was repeated after 2 weeks to allow for comparisons of the insulin and glucose responses after a repeated bout of similar exercise.

During the first visit, participant mass and height were measured and a familiarisation to the maximal voluntary contraction (MVC) and counter-movement jump (CMJ) and perceived soreness were performed to asses muscle function post eccentric exercise. The following visit took place a week after the last familiarisation session and formed the control trial for the study and included baseline measures of serum CK activity, insulin and glucose responses to an OGTT. Participants subsequently completed 2 bouts of squat exercise. The initial bout (SE1) and the repeat bout (SE2) of 5 sets of 20 repetitions were separated by 2 weeks. Serum CK activity, MVC, CMJ and perceived soreness were assessed pre, 24, 48 and 96 hour post eccentric exercise. The circulating insulin and glucose response to an OGTT were measured 48 hours after each bout of squat exercise.

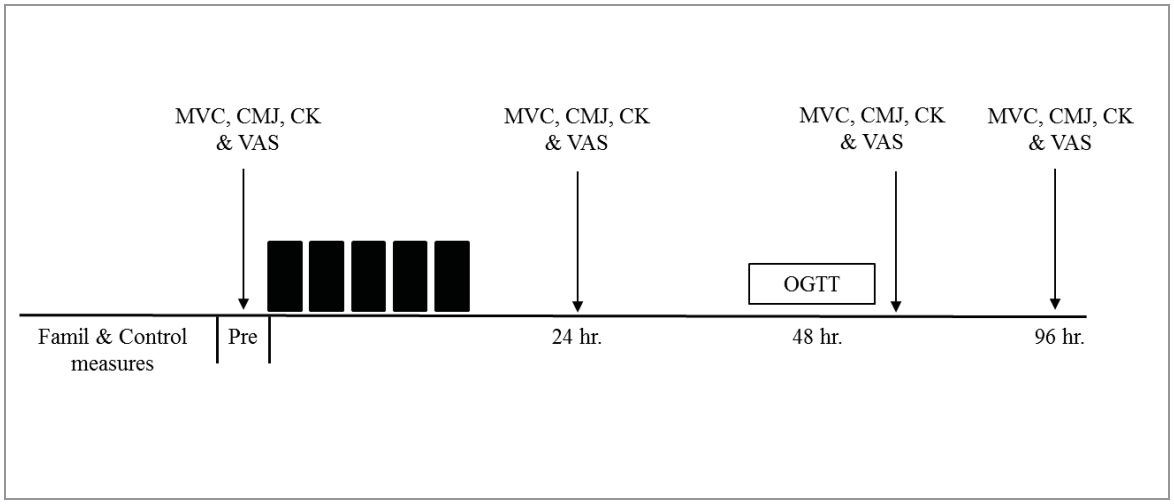

Figure 5.1. Schematic presentation of the experimental protocol. Indicating, eccentric exercise (black boxes), measures of serum CK, MVC, CMJ and VAS that were conducted post eccentric exercise at 24, 48 and 96 hours.

Eccentric exercise protocol

The participants performed 100 barbell squats performed as 5 sets of 20 repetitions. The load on the barbell corresponded to 30% of each participant's body mass. They began the movement with the barbell resting on their shoulders, body erect, legs fully extended (knee = 180°) and toes pointing forward. The movement consisted of an eccentric action of the knee extensors to lower the barbell to a knee angle of less than 90° and a concentric action to raise barbell to the starting position. Since this form of exercise was unfamiliar to all participants, the load was moderate to ensure correct technique and the movement was slow and careful to avoid injury. The volume of the exercise was high and the amplitude of the stretch large, as defined by the depth of the squat exercise and with participants being instructed to take their thighs below parallel on the descending phase of the squat. These factors have been shown to induce muscle damage (Talbot and Morgan, 1998).

Oral glucose tolerance test

In all three trials participants undertook an OGTT at the 48 hour time point, following 10–12 hours overnight fast. A fasting blood sample was drawn, then following ingestion of a 75g oral glucose load (Dextrose, Murdoch Manufacturing Ltd, Christchurch, New Zealand) mixed with 400ml of water over a 5 minute period, venous blood was collected at 15, 30, 45, 60, 90 and 120 minutes via an indwelling cannula inserted into a vein in the antecubital fossa, which was secured by tape and kept patent by flushing with saline (0.9% sodium chloride). The first 2ml of blood withdrawn was always discarded to avoid dilution of the sample by the saline. Following this, a further 7ml sample was drawn. Blood was analysed for serum glucose and insulin concentrations to ascertain glucose tolerance and relative insulin action. Each participant was instructed to maintain the same dietary and exercise patterns the evening before each test and over the 3 days prior to the test. To ensure compliance, participants completed a questionnaire detailing pre-session information about their diet and lifestyle patterns and completed a dietary record for 1 week prior to commencement of the study.

Blood sampling

Blood samples were placed on ice for 10 minutes before being centrifuged (Eppendorf, Hamburg, Germany) at 2,000 – 3,000g for 10 minutes at 4°C. Separated serum was aspirated into 500µl aliquots and frozen at -80°C for later analysis of insulin and glucose concentrations and CK activity. Serum concentrations of insulin were measured in duplicate (Morgan and Lazarow, 1963) using a commercially available human insulin radioimmunoassay kit (Linco Research, St. Charles, MO). Serum glucose levels were measured in duplicate by hexokinase method (Peterson and Young, 1968, Schmidt, 1961). Serum CK activity was measured by enzymatic method using reverse action (Hørder et al., 1991, Klauke et al., 1993).

Area under the curve

Area under the curve for insulin and glucose were calculated using the trapezoid method (NCSS 2007, Statistical & Power Analysis Software, Utah, USA) and expressed as either $\mu U \cdot ml^{-1} \cdot 120min$ or $mmol \cdot L^{-1} \cdot 120min$ respectively. The baseline for

determination of the insulin and glucose AUC calculations was set at fasting levels. Use of the OGTT, along with calculation of the AUC for insulin, has been shown to be a suitable surrogate measure of insulin resistance (Yeni-Komshian et al., 2000).

Maximal voluntary contraction

Maximal voluntary isometric force at approximately 90° knee flexion was assessed using and adapted chair and strain gauge system. In an adjustable straight-backed chair, participants were firmly secured into the chair with a belt passed around the waist to secure the pelvis. An inextensible taut strap was passed around the ankle at the level of the lateral malleolus. The strap was connected to a load cell (Sensortronics Covina, California, USA), which was connected to a transducer (PowerLab 4/25, ADInstruments) and the forces were recorded and displayed on an analogue to digital data software package (Chart for Windows, ADInstruments, Sydney, Australia).

Countermovement jump performance

All jumps were performed on a Kistler force plate (Kistler Instruments Corp, Amherst, NY, USA) and data was analysed using the Kistler BioWare® software (version 3.2.6.104). Centre of mass (COM) assumed that the jumper's position on the platform was the same at take-off and landing. To ensure that this was true, participants were instructed to place their hands on their hips and to keep their body erect throughout the jump. Upon landing, contact was initially made with the toes, while knees were fully extended to ensure the body positions at take-off and landings were identical. The participants started from an erect standing position with knees fully extended (knee = 180°). On the verbal command "Go", the participant made a downward countermovement to a self-selected starting position, with the knee ≤ 90° and then jumped vertically for maximum height in one continuous movement.

Perceived muscle soreness

The same scale to assess perceptions of muscle soreness was used as described in section 3.3. Measures were taken prior to the each trial commencing and then 24, 48 and 96 post eccentric exercise. Soreness was rated while participants held an un-weighted squat position for approximately two seconds and marked their perception of soreness on the scale. This procedure has previously been used successfully to evaluate soreness (Marginson et al., 2005).

Statistical analyses

All statistical analyses were performed using PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL, USA). All data are reported as the mean ± SD. Before carrying out the statistical analysis the residuals in the ANOVA were examined for a normal distribution through investigation of a histogram and a normal-plot. If residuals were considered not to be normally distributed, data were log-transformed and residuals were investigated again. After log-transformation, residuals were considered to be normally distributed and thus for these measurements log-transformed data were used in the subsequent statistical analysis. All measures were compared using a two-way ANOVA (trial × time) with repeated measures. Values from ANOVA were assessed for sphericity. On discovery of a significant *F* test, pair-wise comparisons were identified using Tukey's HSD *post hoc* procedure. The small sample sizes in this study were due to a shortage of relevant prior published work to calculate an Effect Size.

5.4 Results

Performance measures and markers of damage

There were no significant differences in the isometric MVC of the knee extensors across the two trials (P = 0.214). A main effect of trial was observed (P = 0.029) for CMJ performance (Table 5.1). An interaction time × trial (P = 0.022) was also observed. Post hoc testing indicated that the significant difference between initial and repeat bouts lay at the 24 hour time post squatting. A main effect of trial was observed (P = 0.000) for ratings of perceived soreness among participants indicating that the SE1 trial brought about greater sensations of soreness than the SE2. A main effect of time was also observed (P = 0.000), indicating that both trials showed increased levels of soreness as the trial progressed.

	SE1				***SE2***			
	Pre	**24hr**	**48hr**	**96hr**	**Pre**	**24hr**	**48hr**	**96hr**
MVC (Nm)	621 (226)	517 (205)	524 (223)	575 (191)	636 (229)	568 (179)	548 (193)	570 (200)
CMJ (cm)	39.9 (4.6)	36.6*† (4.0)	37.0* (5.3)	38.6 (4.6)	39.9 (4.2)	39.0 (5.4)	39.2 (4.9)	39.2 (5.1)
VAS (cm)	0 (0)	5.4*† (0.8)	7.1*† (0.5)	5*† (0.9)	0 (0)	4† (0.5)	4.7 (0.7)	2.9† (0.6)

Table 5.1. Measures of knee extensor muscular strength, countermovement jump performance and perceived muscle soreness following an initial (SE1) and repeat bout (SE2) of eccentric exercise (n = 8). Data are means (SD). *****Indicates significantly different from repeat bout. †Indicates significantly different from pre-measure (P < 0.05).

Creatine kinase

A significant main effect for time was revealed indicating that serum CK activity increased (Figure 5.2) during the first 24 hours following both bouts of squatting exercise ($P = 0.030$). There was a near significant main effect of trial ($P = 0.053$) and a significant interaction trial × time interaction ($P = 0.006$) showing that the 4 day serum CK response to squatting was different between SE1 and SE2. Post hoc testing revealed that the interactions lay between the control trial, SE1 and SE2 when comparing the 24 hour measures to the pre-measures.

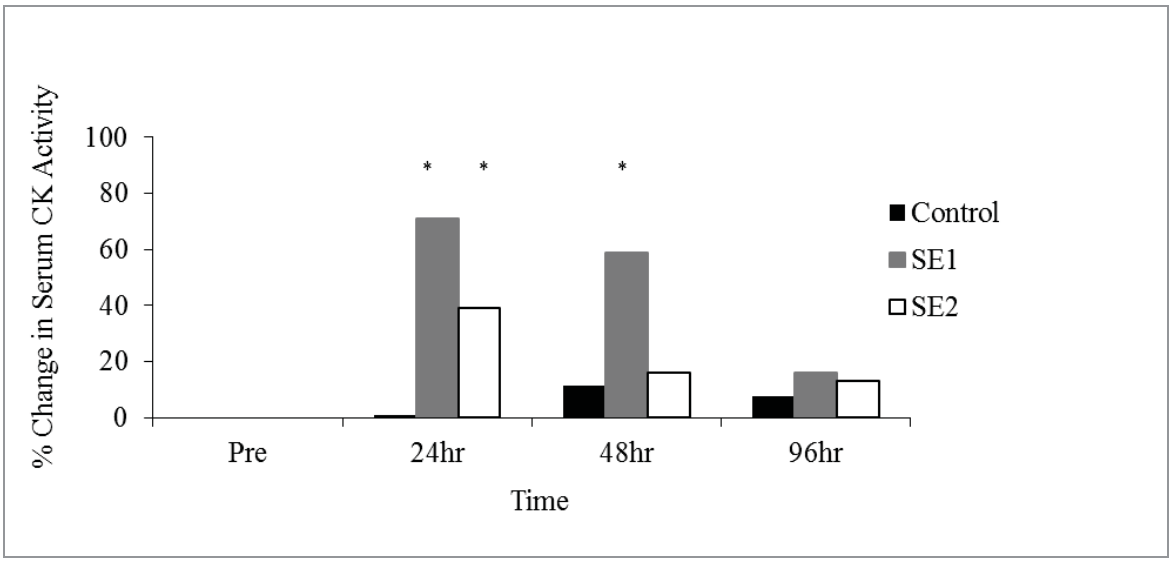

Figure 5.2. Percentage change in serum CK activity in response to the initial squat exercise (SE1) and the repeat bout (SE2) (n = 8). Data are means (SD). *Indicates significantly different from pre-measure ($P < 0.05$).

Glucose

Fasting glucose concentrations were not significantly different between the three trials ($P = 0.834$). The fasting levels of blood glucose were also not significantly different across the time course of the three trials ($P = 0.488$). A main effect for time was evident following the OGTT ($P = 0.000$) with the peak blood glucose appearance occurring at 30 minutes post glucose solution consumption in all three trials (Figure 5.3). However, it was revealed that no significant differences in blood glucose concentrations between any of the trials ($P = 0.905$) during OGTT. Glucose AUC was

not significantly different (P = 0.742) between the three trials (mean ± SD: 714 ± 114; 700 ± 114 and 711 ± 161 mmol.L^{-1}.120min for control; SE1 and SE2 respectively).

Insulin

Fasting insulin concentrations were not significantly different between the three trials (P = 0.968). The fasting levels of blood insulin were also not significantly different across the time course of the three trials (P = 0.076). A main effect for time was evident following the OGTT (P = 0.000) with the peak blood insulin appearance occurring at 30 minutes post glucose solution consumption in the control and SE1 trials but occurring at 45 minutes in SE2 (Figure 5.3). However, three-way ANOVA revealed no significant differences in insulin concentrations between any of the trials (P = 0.762). Insulin AUC was not significantly different (P = 0.955) between the three trials (mean ± SD: 7267 ± 2869; 7553 ± 3837 and 6872 ± 2598 μU.ml^{-1}.120min for control; SE1 and SE2 respectively).

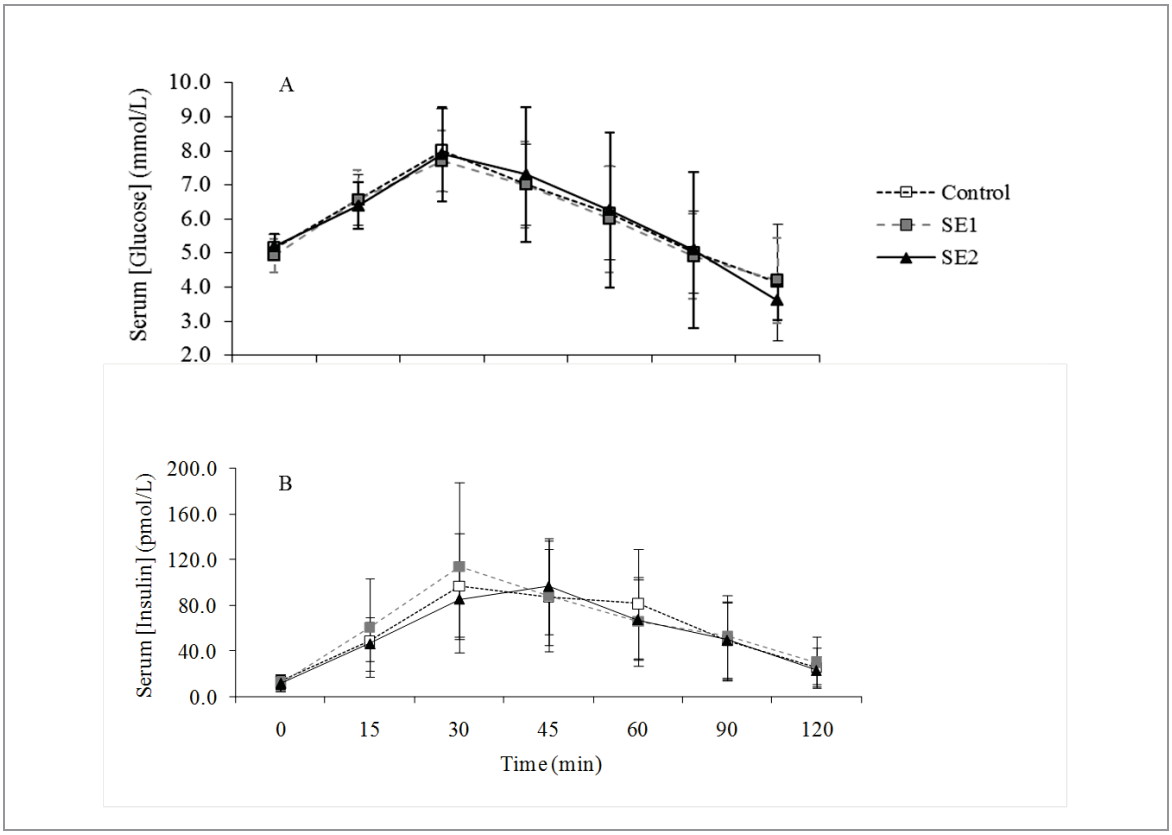

Figure 5.3. (A) Glucose and (B) Insulin response to an oral glucose-tolerance test (OGTT) administered at baseline (control) and 48 hours after two bouts of squat exercise (SE1 and SE2) (n = 8). Data are means (SD).

5.5 Discussion

The major findings of this study were that the damaging exercise protocol employed did not produce alterations to insulin and glucose responses to an OGTT, indicative of short term insulin resistance. Also, there was no difference in the response to the OGTT after an initial bout of exercise that was eccentrically biased and a second bout of eccentric exercise performed 2 weeks later.

Participants' functional and perceptual responses to a novel bout of eccentrically biased exercise in the current study was in general agreement with those of previous investigations (Byrne and Eston, 2002, Byrnes et al., 1985, Clarkson and Tremblay, 1988, Newham et al., 1983b, Nosaka et al., 2001). The initial bout of eccentric exercise resulted in elevations in muscle soreness (Table 5.1), as well as prolonged decrements in CMJ performance (Table 5.1). The CK response to both bouts of exercise was also similar to those reported in the literature. The perturbations in soreness, CMJ, MVC and CK indicate that the initial bout of squat exercise induced a mild degree of muscle damage.

The current study revealed a repeat bout effect after SE2 for CK, soreness and CMJ, as has been previously demonstrated (Byrnes et al., 1985, Clarkson and Tremblay, 1988, Newham et al., 1987, Nosaka et al., 2001). The repeat bout effect was evidenced by reduced muscle soreness 48 hours after SE2 (Table 5.1), enhanced recovery of CMJ (Table 5.1) and attenuation of the CK response (Figure 5.3). The deficits of MVC were not significantly reduced in the current study after either the SE1 or the SE2.

The alteration in the insulin response following an acute bout of eccentric exercise has been studied via a standard OGTT (Sherman et al., 1992). Following a downhill treadmill run to induce EEIMD, the authors demonstrated that it resulted in peak insulin and insulin AUC responses higher than control values. This is contrary to the findings of the current study where there were no alterations to the insulin response following OGTT 48 hours after SE1 in comparison to the control trial. The blood glucose response to the OGTT was not altered, suggesting no alteration in glucose tolerance in either the present study or that conducted by Sherman, et al. (1992). Sherman et al (1992) suggested that a greater amount of insulin was required to maintain glucose

homeostasis after eccentric exercise, though this cannot be confirmed by the present study. Kirwan et al. (1992) hypothesised that decreased insulin 'effectiveness' resulted from disruptions to binding of insulin to its receptor, reductions in the availability of glucose transporters, or alterations in the intracellular glucose disposal pathway.

It should be noted that the insulin and glucose response to a standard OGTT (as in the current study) is only a surrogate, indirect measure of insulin sensitivity. Direct measurement of insulin sensitivity would require methods such as the euglycemic-hyperinsulinemic clamp technique (Defronzo et al., 1979), whereby the amount of glucose infused to maintain a set blood glucose level is indirectly proportionate to the degree of insulin resistance.

In the current study, 48 hours after SE1, peak insulin during the OGTT was not elevated compared with the control trial. In contrast to the findings of Green et al. (2010), but in line with the findings of Sherman et al. (1992), peak glucose and glucose AUC were not significantly elevated in response to SE1. It is important to note that Green et al. (2010) used female participants who were considerably lighter in mass and less lean than the male participants used in the current study. Participants in the present study received glucose in amounts equal to 0.96 ± 0.12 g/kg lean body mass, whereas participants in the Green et al. (2010) study received glucose in amounts approximating 1.6 g/kg lean body mass. Increased provision of glucose, combined with a greater percentage of body fat, could partially explain the discrepancy between studies.

To our knowledge there is little published research that investigates the RBE and its influence on both insulin and glucose activities occurring following eccentric exercise. The results of the current study do not demonstrate any change to the insulin or glucose response (during an OGTT following an initial bout of eccentric exercise) or any attenuation following a repeat bout of eccentric contractions (Figure 5.3). No elevations in peak insulin or glucose and the respective AUC were evident in comparison to control trials after either SE1 or SE2. Therefore, this would indicate that neither of the two bouts of squat exercise had altered the circulating level of insulin or the uptake of glucose from that evident in the control trial. Therefore, as markers of damage increased (perceived soreness, CK activity) across SE1, the levels of insulin and glucose circulating the body remained stable. An explanation may be due to the fact that the

initial eccentric bout appeared to induce only a mild degree of muscle damage as indicated by the low serum CK activity (120 ± 65 vs. 408 ± 378 IU/L at its peak 24 hours following SE) - this was lower than that reported by others (Davies et al., 2008, Davies et al., 2009).

Measurement of MVC is the most widely utilised method in determining the extent of muscle damage (Warren et al., 1999), due to its high reliability (intraclass correlation coefficients 0.85). If measurements are always made at the same joint angle MVC torque is a very valid tool (Kellis and Baltzopoulos, 1995, Abernethy et al., 1995). However, the results of the MVC from this study could have arisen as a result of different motor unit recruitment patterns post exercise (Sale, 1987). This is surprising because it has been shown that performing only a small quantity of eccentric contractions confers a significant protective effect in regards to indices of strength, soreness and CK activity (Clarkson and Tremblay, 1988).

A number of mechanisms have been suggested to explain the adaptations that occur following eccentric exercise which confer the prophylactic effect. These mechanisms have been classified into neural, connective-tissue and cellular theories (McHugh, 2003, McHugh et al., 1999). The proposition that neural adaptations are responsible suggests that any initial damage will stimulate additional motor-unit activity (Komi and Buskirk, 1972), recruitment of slow-twitch fibers and generate better motor-unit synchronization in subsequent eccentrically biased exercise (Golden and Dudley, 1992). Further, any force encountered during a repeated bout of eccentric contractions is distributed over a larger area of muscle fibres (Nosaka and Clarkson, 1995). Connective-tissue adaptation describes increases in the amount of intramuscular connective tissue present after an initial bout of eccentric exercise (Lapier et al., 1995) and the remodelling of intermediate filaments (Fridén et al., 1983a). Cellular-adaptation suggests that cell membranes are strengthened (Clarkson and Tremblay, 1988) and weak fibres that were damaged are removed from the injured site (Mair et al., 1995) and the addition of sarcomeres in series (Lynn and Morgan, 1994). The addition of sarcomeres in series as the rapid adaptation response to an initial bout of eccentric exercise is now the much more accepted mechanism. This mechanism is based on the predictions of Morgan (1990), which suggests that the repair process will result in an increase in the number of sarcomeres in series, reducing sarcomere strain during a repeat bout and therefore

reducing muscle damage. Direct evidence has been provided of the addition of sarcomeres following eccentric exercise in animal models (Lynn and Morgan, 1994, Lynn et al., 1998). Indirect evidence in human experiments has been demonstrated by the rightward shift in the length-tension relationship following recovery from an initial bout and this was attributed to longitudinal addition of sarcomeres (Brockett et al., 2001).

In conclusion, in the current study, the squatting eccentric biased exercise resulted in mild muscle injury. A second bout of the same eccentric exercise performed two weeks later was associated with a lesser degree of muscle injury. This study neither found evidence that elevated insulin responses were observed after a novel bout of eccentric exercise, nor that a later bout of eccentric exercise attenuated the responses. Further studies are warranted to determine whether these phenomena are related to protocol employed by this study.

6. Effect of eccentric versus concentric training on metabolic measures following eccentric exercise induced muscle damage

6.1 Abstract

Repeated eccentric based exercise initiates a protective adaptation so that replicating the same eccentric exercise at a later date results in reduced symptoms of damage compared to the initial bout. Following an acute initial bout of eccentric exercise, an increase in the P_i / PCr occurs but it is not known if this indirect measure of intracellular metabolism is attenuated by repeated exposure to eccentric exercise. Using ^{31}P-MRS the purpose of this study was to examine the effect of a bout of EEIMD on intramyocellular phosphate metabolism in skeletal muscle, which had been concentrically (ConTr) versus eccentrically (EccTr) trained. Five males and one female (35 ± 7 yrs mean ± SD; 174.5 ± 7.2 cm and 70.9 ± 7.1 kg body mass) performed six training sessions on an isokinetic dynamometer (5 x 10 reps) on each leg; one leg was trained eccentrically and the other concentrically. Following the training period participants undertook a bout of eccentrically biased exercise, which had previously been shown to induce EEIMD. This involved 300 unilateral, maximal, isokinetic, eccentric contractions (EE) ($30°.s^{-1}$) using the quadriceps of both legs on an isokinetic dynamometer. Unlocalised ^{31}P-MRS was used to assess resting [PCr], [P_i], [ADP], pH and P_i / PCr. Significant increases in isometric MVC ($P < 0.05$) were evident following both training conditions (pre 231 ± 43Nm to post 265 ± 45Nm; pre 235 ± 58Nm to post 276 ± 64Nm in ConTr and EccTr respectively), though the ConTr leg experienced a significant decrease in MVC after EE (276 ± 64 to 197 ± 21Nm). A main effect of trial was evident for muscle volume, which was significantly greater following EE in the ConTr leg compared to the EccTr leg ($P = 0.045$). The P_i / PCr were above 0.19 in both ConTr and EccTr at both time points which was consistent with EEIMD in both legs. However, there were no significant differences in resting [PCr], [P_i], [ADP], pH or P_i / PCr between ConTr or EccTr conditions following EE (all $P > 0.05$). These findings indicate that increases in skeletal muscle phosphate metabolism typically associated with muscle damage are not attenuated by prior exposure to repeated bouts of EE, and decrements in force generating capacity of muscle may be mediated by more central factors.

6.2 Introduction

Unaccustomed eccentric exercise of a high intensity has been shown to alter skeletal muscle structure and function. Alterations to the morphology of muscle include disruption of the cytoskeleton, sarcolemma and T-tubules (Fridén and Lieber, 2001). Sarcolemmal integrity is compromised, which results in an efflux of intramyocellular proteins and enzymes into the circulation (Evans et al., 1986) and an influx of extracellular Ca^{2+} into the sarcoplasm (Duan et al., 1990).

Several studies have shown that there is a pronounced training effect to repeated eccentric training which is characterised by attenuation of markers and symptoms of EEIMD including reduced sensations of soreness, impairment of muscle function and efflux of enzymes into the circulation (Balnave and Thompson, 1993, Newham et al., 1987). In contrast, periods of concentric training do not confer the same protective effect (Ploutz-Snyder et al., 1998, Schwane and Armstrong, 1983, Whitehead et al., 1998). This adaptive response (Brown et al., 1997a, Eston et al., 1996, Golden and Dudley, 1992, Mair et al., 1995, Nosaka and Clarkson, 1995) can be observed even before full recovery from EEIMD has occurred, such that further physical stress on the affected muscles in the early (~ 24 hour) recovery phase does not exacerbate or delay the rate of recovery (Chen and Hsieh, 2001, Nosaka and Newton, 2002b).

Measurement of limb circumference to track swelling caused by eccentric exercise have been documented in numerous studies (Clarkson et al., 1992, Howell et al., 1993, Rodenburg et al., 1994). The results typically show that the circumference increases immediately after exercise. This acute swelling subsides quickly, followed by a gradual rise to a maximum that occurs 1 to 4 days after eccentric exercise. Evidence has been provided that swelling, via measurement of circumference, is reduced in muscle that has undertaken a prior bout of eccentric exercise (Nosaka et al., 2001). Though relatively simple and inexpensive to collect, measures of swelling are susceptible to poor reliability, and do not necessarily track well with other indicators of EEIMD (Cleak and Eston, 1992b, Warren et al., 1999). Magnetic resonance imaging measurements of muscle volume provide a more precise and reproducible estimate of total muscle swelling.

A consequence of structural alterations following unaccustomed exercise include the reduced ability of skeletal muscle to voluntarily generate force (Clarkson et al., 1992). Studies of single muscle fibres have shown that impaired force generation following EEIMD is caused by reduced Ca^{2+} release (Balnave and Allen, 1995, Ingalls et al., 1998, Warren et al., 1993). Further, it has been established that an increase in [P_i] reduces cross-bridge force and Ca^{2+} sensitivity of the myofilaments (Cooke and Pate, 1985) and may be a contributor to the reductions in voluntary force generation observed. It has already been established in Chapter 4 and by others (Aldridge et al., 1986, McCully et al., 1988) that following an acute bout of eccentric exercise [P_i] increases and remains elevated for around 48 hours.

An increase in the resting P_i / PCr ratio occurs with EEIMD and is seen as indicative of structural and cellular damage (McCully et al., 1988, Aldridge et al., 1986). The rise in P_i / PCr has been shown to peak 48 hours after eccentric exercise (McCully et al., 1988, Rodenburg et al., 1994, Zehnder et al., 2004).

The purpose of this study was to examine whether exposure to a short period of eccentric training would confer a prophylactic effect on EEIMD induced alterations in muscle phosphate metabolism. It was hypothesised that eccentric muscle training would attenuate the rise in resting P_i and subsequent increase in P_i / PCr during the 48 hours following eccentric exercise.

6.3 Methodology

Participants

Five males and one female who were not accustomed to eccentrically based exercise volunteered to participate in this study; mean ± SD age, height and body mass were 35 ± 7yr, 174.5 ± 7.2cm and 70.9 ± 7.1kg respectively. None of the participants had a history of muscle or metabolic disease. All participants completed a health screening questionnaire and provided written informed consent. Participants were asked not to participate in strenuous exercise for the preceding two weeks and for the duration of the protocol. The University's Research Ethics Committee approved all procedures prior to the start of the investigation.

Experimental overview

To test the hypothesis a one legged model was employed and implemented in a randomised balanced order. This model enabled circulating factors to affect both legs equally and thus not confound measurement of the direct/muscular effects of eccentric training on muscle metabolism. Participants completed a 2 week period of training comprising 3 sessions per week with at least 1 day of rest in between sessions. During each session the participants were required to complete 100 maximal contractions on an isokinetic dynamometer (Biodex Medical Systems, New York, USA). The 100 maximal contractions were broken down with 50 contractions to be completed by each leg and split into eccentric only contractions on one leg (EccTr) and concentric only contractions on the other control leg (ConTr) (Figure 6.1). The determination of which leg completed the EccTr and ConTr contractions were randomised and balanced.

Prior to and following the training 5 maximal isometric, concentric and eccentric contractions of the quadriceps muscles were completed as tests of muscle performance. Isometric tension was measured at a knee angle of 75° (1.31 rad). Concentric and eccentric torque was measured at an angular velocity of $30^{\circ}\ s^{-1}$ ($0.52\ rad\ s^{-1}$) (MacIntyre et al., 1996). Absolute peak torque over 5 contractions was recorded. Each measure was separated by 2 minutes of passive recovery. Forty eight hours following the

completion of the training period, a bout of maximal voluntary EE was undertaken on each leg. The tests of muscle performance were completed 48 hours following the EE.

Magnetic resonance spectroscopy was also utilised at both 24 and 48 hours post EE to quantify muscle metabolites at rest. The 24 and 48 hour time points were chosen because these coincide with the greatest change in the ultra-structure of the muscle fibre and pronounced feelings of muscle soreness (Clarkson et al., 1992) and rather than look at pre- post differences it was of greater importance to capture the temporal alterations following eccentric exercise. Participants were scanned using unlocalised ^{31}P-MRS to derive muscle [TCr], [PCr], [P_i] and [ATP] of the quadriceps muscles. These measures were made for the purpose of confirming EEIMD (McCully et al., 1992), because this could not be confirmed with circulating markers (e.g. CK) using the study design employed. Further, these measures were used to compare the level of damage between ConTr and EccTr. No dietary restraints were placed on the participants. However, a record of what was eaten over the course of the experiment was taken to confirm that participants followed a eucaloric diet. Participants were instructed to avoid exercise other than walking during the 48 hours after EE.

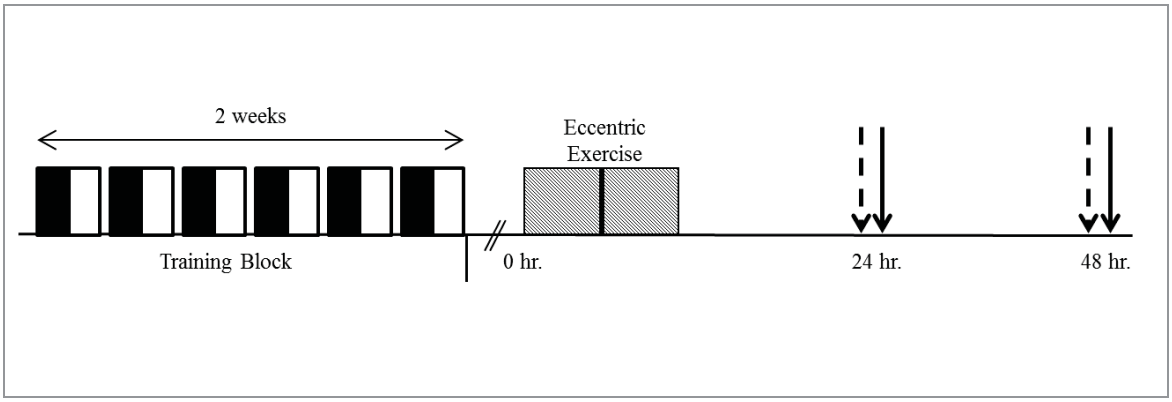

Figure 6.1. Schematic presentation of the experimental protocol. Indicating, EccTr (black box) and ConTr (white box) bouts, EE protocol (dashed box) and measures of resting ^{31}P-MRS (dashed arrow) and MVC (solid arrow). MRS and MVC were conducted 24 and 48 hour post eccentric exercise.

Eccentric exercise protocol

The set-up of the isokinetic dynamometer was identical for the training bouts and the experimental exercise. This set-up replicated the design for the study conducted in Chapter 4 (see section 4.3).

Spectral analyses and muscle volumes

Axial dual-echo images were acquired using the Q body coil (slice thickness 6 mm; slice gap 30 mm) from below the knee joint to above the hip joint. Resting ^{31}P-MRS measurements were acquired as described previously in Chapter 4 (see section 4.3). Muscle volumes were calculated as described previously in Chapter 4 (see section 4.3).

The ^{31}P-MRS spectra were analysed using a JAVA-based magnetic resonance user interface (jMRUI version 3.0, EU Project) and the relative amplitudes measured for PCr, P_i and α-, β-ATP and γ-ATP using quantitation based on quantum estimation (QUEST). All signals were fitted without pre-processing or phase correction. T1 saturation effects were corrected relative to the initial resting spectra. Amplitudes were corrected for proton decoupling and NOE. Concentrations of PCr and P_i were then calculated relative to β-ATP assuming a constant [ATP] of 8.2 mmol L^{-1} (Taylor et al., 1986). The chemical shift between PCr and P_i was used to measure muscle pH (Moon and Richards, 1973). Total basal muscle creatine was calculated assuming PCr = 0.61 x TCr (Rico-Sanz et al., 1998). The free [ADP] was calculated as:

$$[ADP] = [\sum ATP](([\sum TCr]/[\sum PCr]) - 1)/(K_{CKapp}[H^+])$$

where K_{CKapp} is a function of the binding of H^+, K^+ and Mg^{2+}, which in turn is a function of pH (Kemp et al., 2001).

Statistical analyses

All statistical analyses were performed using PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL, USA). All data are reported as the mean ± SD. Three-way repeated measures ANOVAs were conducted to determine any differences in muscle metabolites between conditions (trial × time). Muscle volume measurements were compared using a two-way ANOVA. Statistical significance was accepted at $P < 0.05$. The small

sample size in this study is based on significant effects found on P_i and P_i / PCr in Chapter 4 with the same sample size and, of course, this minimised expenditure. But there was confidence that a significant effect could be detected.

6.4 Results

Total work done during training and experimental protocol

The total work done during the training phase was significantly different between contraction types (P = 0.007). The EccTr resulted in a larger amount of work being carried out by the contracting muscle compared to ConTr (56 ± 8 vs. 46 ± 8 kJ respectively).

There was no significant difference (P = 0.571) in the amount of energy expended by either leg the ConTr or EccTr leg (44 ± 13 vs. 45 ± 10 kJ respectively) during the EE bout. Following EE, all subjects reported 'extreme' delayed soreness in the ConTr leg compared with the EccTr leg, throughout the post EE time frame.

Skeletal muscle function

A main effect for time was observed for isometric MVC (P = 0.037) with an increase in force evident in both legs following training. Post hoc testing revealed that only the ConTr leg experienced significant decrements in force after EE. A main effect for trial (P = 0.016) was evident for eccentric MVCs with a larger post training gain in MVC in the EccTr leg and no decrease following EE in the EccTr leg. Also, an interaction effect of time × trial (P = 0.047) was observed with the differences being between the post training and post damage time points and the ConTr and EccTr legs. There were no significant changes in concentric MVC in either training condition (P = 0.365; P = 0.415 time and trial respectively).

	ConTr Peak Torque			*EccTr Peak Torque*		
	Pre	**Post Training**	**Post EE**	**Pre**	**Post Training**	**Post EE**
Iso (Nm)	231 (43)	265 (45)*	197 (21)*^	235 (58)	276 (64)*	228 (60)
Con (Nm)	211 (46)	261 (64)	226 (67)	237 (40)	249 (60)	239 (54)
Ecc (Nm)	241 (74)	322 (65)*	254 (65)^	264 (79)	334 (65)*#	336 (66)*#

Table 6.1. Maximal voluntary contractions for ConTr and EccTr legs (n=6). Isometric (ISO), concentric (CON) and eccentric (ECC) force measurements made before and after training and 48 hours following 300 eccentric contractions of the *quadriceps* under concentric (ConTr) and eccentric (EccTr) conditions. Data are means (SD). Differences between time points were evaluated by post-hoc pairwise comparison. *Indicates significantly different from pre- training value. ^Indicates significantly different from post-training value. #Indicates significantly different from ConTr ($P < 0.05$).

Quadriceps muscle volume

A main effect of trial was evident for muscle volume, which was significantly greater following EE in the ConTr leg compared to the EccTr leg ($P = 0.045$). There was a significant difference across time with volume increasing at 48 hour compared to 24 hour measures ($P = 0.015$).

Skeletal muscle ^{31}P-MRS

There were no significant main effects for trial or time ($P = 0.650$; $P = 0.369$ respectively) for resting [PCr]. There were no significant main effects for trial or time ($P = 0.212$; $P = 0.797$ respectively) for resting [P_i]. There were no significant main effects for trial or time ($P = 0.344$; $P = 0.384$ respectively) for resting ratio of P_i / PCr. There were no significant main effects for trial or time ($P = 0.906$; $P = 0.963$ respectively) for resting [ADP]. There were no significant main effects for trial or time ($P = 0.886$; $P = 1$ respectively) between legs for resting pH (Table 6.2).

	ConTr 24hr	*ConTr 48hr*	*EccTr 24hr*	*EccTr 48hr*
Muscle volume (cm^3)	1122 (265)	1184 (243)*	1104 (233)#	1098 (245)#
[PCr] *(mM)*	19.30 (4.87)	19.37 (3.35)	18.25 (2.12)	19.44 (2.92)
[P_i] *(mM)*	4.03 (1.03)	4.03 (1.17)	3.66 (0.56)	3.73 (0.62)
P_i /PCr	0.21 (0.04)	0.21 (0.04)	0.20 (0.03)	0.19 (0.03)
[ADP] (µM)	42.7 (3.9)	43.4 (4.8)	43.3 (2.9)	42.5 (4.4)
pH	7.00 (0.04)	7.01 (0.05)	7.01 (0.03)	7.00 (0.05)

Table 6.2. *Quadriceps* muscle volumes and resting metabolite concentrations in ConTr and EccTr legs (n=6). Data are means (SD). *Indicates significantly different from prior measure. #Indicates significantly different from ConTr ($P < 0.05$).

6.5 Discussion

The purpose of this study was to examine whether EEIMD was associated with alterations in skeletal muscle metabolism *in vivo* and also whether exposure to a short period of eccentric exercise training would confer a prophylactic effect on these alterations.

Previously in this thesis (Chapter 4) it was shown that following an acute bout of eccentric exercise which induced EEIMD, there was an elevation of resting [P_i] in comparison to non-damaged muscle that had undertaken a bout of matched work in a concentric manner. The previous study also revealed an increase in the ratio of P_i / PCr, a measure that is utilised as an unspecific marker of muscle damage (McCully et al., 1988). In the present study, following the damaging bout of EE, both eccentrically and concentrically trained legs were found to have similar resting [P_i]. The results also show that muscle exposed to a period of eccentric contractions (EccTr) has a similar ratio of P_i / PCr as muscle that has been exposed only to prior concentric contractions (ConTr). This suggests that using this unspecific marker of muscle damage, no attenuation is evidenced in EccTr muscle utilising the training regime in this study. The lack of differences in resting levels of [PCr] in muscle exposed to prior eccentric contractions would indicate that both legs have a similar availability of high energy phosphates for contraction and that no protective effect was offered to the EccTr leg.

Studies of skinned and intact skeletal muscle fibers have shown that increased [P_i] is associated with decreased capacity for isometric muscle tension (Chase and Kushmerick, 1988, Cooke and Pate, 1985). This decrease in isometric muscular tension with a concomitant increase in [P_i] has been attributed to the effect of P_i on cross-bridge cycling. Increased levels of P_i have been acknowledged to reduce the number of cross bridges attached in the strongly bound state (Cooke et al., 1988). There was no reduction in the force generating capacity of the muscle exposed to EccTr following EE even though the resting [P_i] was similar in both conditions following the EE. Although no pre-bout measure was performed, an increase in [P_i] can be identified because values of P_i / PCr were ≥ 0.19, which is higher than P_i / PCr in undamaged muscle reported in Chapter 4 (Section 4.4) and in literature (McCully et al., 1992); while at the same time,

it is known that an increase in P_i / PCr is attributed to an increase in [P_i] rather than a reduction in [PCr] (Aldridge et al., 1986). This may suggest that a role for P_i as a causative agent in reducing force generation is unlikely and that there may be an additional factor present to inhibit force generation. This factor cannot be a morphological change alone, since morphological degeneration has been found to be present for more than temporal conditions in this study (Friden et al., 1983).

The findings of Chapter 4 showed a clear difference in [P_i] between the eccentrically exercise leg and the concentrically trained leg (Section 4.3, Table 4.1). However the results in the current study show the [P_i] following EE in both the ConTr and EccTr conditions to be lower than that previously shown in Chapter 4. This is not indicative of an attenuation in the [P_i] following either training condition. But is more likely a function of the utilisation of QUEST as our post-processing quantification method over AMARES. When compared with AMARES, the QUEST method has less 'overfitting' of small resonances including the ATP peaks and therefore leads to slightly lower [PCr] and [P_i]. Importantly, the ratio of P_i / PCr in this study was similar to that in Chapter 4 and confirmed a lack of any protection to the muscle that was EccTr.

Previously observed increases in the P_i / PCr ratio after eccentric exercise have led to the assumption that this reflects an increased rate of cellular metabolism for repair processes (Lund et al., 1998b, Lund et al., 1998a, McCully et al., 1992, McCully et al., 1988, Rodenburg et al., 1994). This increase in cellular metabolic rate may reflect an increase in ion transport pumping activity to compensate for leakage of the membranes, or as a result of increased extracellular P_i in response to the elevation of extracellular fluid. This would mean the P_i / PCr ratio should follow the same time course as CK efflux and edema, since sarcolemmal disintegration is the central factor in both. Therefore, it could be that an increase in cell metabolism was a more likely reason for the elevation of the P_i / PCr ratio.

The functional changes which typically result from prior strenuous eccentric exercise, including attenuation of the decrement in force generation, can be alleviated by previous regular eccentric training (Nosaka and Newton, 2002a). The current data confirm that muscle exposed to regular contractions of an eccentric nature shows an adaptive response, at least in terms of swelling (muscle volume) and functional performance; i.e.

the loss of force in ECC and ISO contractions in the EccTr leg was less pronounced than that of the ConTr leg. Measures of isometric MVC were similar in both conditions, with increases after both bouts of training but a more significant drop, (26% of the post-training measure), in the performance of the ConTr leg following EE. Even though the EccTr leg also exhibited a decrease (17%) in isometric force following EE, this was not significant. There were similar non-significant changes in the generation of concentric MVC, although greater percentage changes were evident in the ConTr leg; a 19% increase after training in comparison to a 5% increase after training for the EccTr leg. The ConTr leg showed a 13% decrease in concentric MVC after EE whilst the EccTr leg only showed a 4% decrease following EE, again indicating some protection from the eccentric training. The changes in the measures of eccentric MVC showed the greatest differences between the two conditions. Whilst the ConTr leg showed a 25% increase in force generation, (compared to 21% in the EccTr leg), it also suffered from a 21% decrement in performance following EE. The EccTr leg showed no decrease in performance following EE. In summary, therefore, these data show that the eccentric training resulted in maintenance of both static (in the form of isometric MVC) and dynamic (concentric and eccentric MVC) function following a damaging bout of eccentric exercise.

The temporal pattern of muscle swelling following EEIMD shows an immediate initial increase in muscle volume with the peak occurring 4 days following the eccentric exercise and a return to baseline over the following days, (Nosaka et al., 2002) or continued to decrease for a period of 4 days (Cleak and Eston, 1992b). Similar temporal patterns for swelling have also been observed by a number of other authors (Zainuddin et al., 2005a, Zainuddin et al., 2005b). Evidence of a further adaptation to the eccentric exercise training was confirmed via magnetic resonance imaging (MRI) measurements of *quadriceps* volume. Namely, repeated exposure to eccentric exercise attenuated the muscle swelling evident following the EE bout in the control condition and can support previous finding of adaptation to EE (Nosaka et al., 2001). MRI was used for this measure because it provides a more precise and reproducible estimate of total muscle swelling compared to measurement of the limb circumference. This outcome is consistent with signs of edema following eccentric contractions of the elbow flexors noted by Shellock et al. (1991) who observed significant swelling via MRI-

measurement of muscle CSA, which peaked 4 days after eccentric exercise (Dudley et al., 1997). The data in this study also confirms that there initial increase in muscle volume following EE, evidenced in these prior results by measures of circumference. However, to our knowledge this is the first study to show that muscle volume is attenuated by a prior period of eccentric training.

Together, these measures of muscle function support the contention that the reduction in the ability to generate force during a subsequent bout of eccentric training limits the increase in damage to the muscle undertaking the exercise (Cleak and Eston, 1992a, Paddon-Jones et al., 2000). It has been indicated that a repeated bout of eccentric exercise completed before recovery of EEIMD from a previous bout does not exacerbate symptoms of muscle damage, and does not alter recovery from muscle damage. This would have been the case in the period of training in the current study. Nosaka et al. (2001) have suggested that this may be a result of a reduced force-generating capacity following a prior bout, but may also indicate that the protective mechanism for the RBE can occur soon after the initial bout of damaging exercise.

It has been suggested that individuals may still continue training while suffering from DOMS without significant risk of further damage (Nosaka and Clarkson, 1995). An explanation of why additional exercise stress before full recovery does not exacerbate muscle damage may be that sarcomere disruption resulting from eccentric damage does not extend across a whole muscle fibre, with myofibrils adjacent to those damaged appearing relatively normal (Connolly et al., 2003). In fact, morphological evidence has demonstrated that not all z-bands from a myofibril are disrupted (Fridén et al., 1983b). In an eccentrically damaged muscle, therefore, additional workload on subsequent days may be distributed to the intact myofibrils and may not affect recovery of the damaged myofibrils.

In conclusion, it is apparent that from the data presented in this study that the metabolic state of skeletal muscle is not affected following exposure to a period of eccentric training. Although we cannot exclude an important role for centrally-mediated fatigue, our results indicate that increases in resting [P_i], may be a contributory factor to the reduced force generating capabilities that are observed following EEIMD.

7. Discussion

7.1 Main Findings

The preceding chapters have studied the metabolic perturbations that occur as a result of EEIMD. The methodologies applied to this study have looked to strengthen the existing literature by providing more valid experimental control to the interventions. Specifically, by employing MRS, it has enabled the observation of metabolic function *in vivo* following EEIMD. The findings point to the stability of high energy phosphates and the potential to maintain a stable short-term energy supply to contracting muscle, which allows the muscle to retain some functionality once it is considered damaged.

The thesis describes three stages in a programme of study investigating the metabolic effects of EEIMD: (1) whole body fuel selection and storage; (2) glycaemic control; (3) tissue specific measures of high energy phosphates following acute and repeat bouts of eccentric exercise.

7.1.1 Whole body substrate utilisation

It was hypothesised in this thesis that there would be a change in substrate utilisation for subsequent bouts of exercise following an acute bout of strenuous eccentric exercise. Previous studies measuring whole body glycaemic control have reported that glucose uptake in muscle considered to be damaged is altered following eccentric exercise (Costill et al., 1990, Doyle et al., 1993, O' Reilly et al., 1987, Widrick et al., 1992). Further investigations have reported that glycogen resynthesis is compromised following a bout of unaccustomed eccentric exercise (Asp et al., 1998, Asp et al., 1995, Costill et al., 1990, Widrick et al., 1992) and logically, this should be reflected in altered substrate selection during subsequent contractile activity. However, these previous studies, which have evidenced decreases in glucose uptake and glycogen resynthesis, all used unexercised muscles as the control (Asp et al., 1998, Costill et al., 1990). However, prior exercise will affect intramuscular substrate concentrations and thus subsequent fuel selection. To understand the role that EEIMD plays on substrate utilisation during subsequent bouts of exercise, Chapter 3 employed a single leg model where the control condition was exercised, but in a concentric rather than eccentric fashion.

The subsequent bout of exercise that was employed in Chapter 3 was one-legged cycling, an activity that is wholly concentric. The choice of this exercise modality was in part due to studies that have shown measures of economy during cycling at fixed loads are not as susceptible to alterations in patterns of movement, such as gait, during locomotion following eccentric exercise (Dutto and Braun, 2004). There is evidence that EEIMD does not alter cycling economy irrespective of the mode of damage employed or the training status of the participants (Gadian, 1992, Gleeson et al., 1995, Moysi et al., 2005, Schneider et al., 2007, Twist and Eston, 2009, Walsh et al., 2001). These studies showed that there were no changes in $\dot{V}O_2$ measures during constant load cycling following the damaging bout of eccentric exercise. This result was corroborated by the findings reported in Chapter 3, which allowed the effects of EEIMD to be isolated using respiratory gas analysis. The method employed to induce damage in this study was a bench stepping exercise which, during the lowering phase of bench stepping, involves eccentric contraction of the knee-extensor and hip-extensor muscles. The propulsive phase of a pedal stroke during cycling (downward phase) also utilises knee extension and hip extension. Therefore, bench stepping is likely to cause EEIMD in the muscle groups primarily responsible for power generation during a pedal cycle in the damaged leg. A number of other studies have utilised this protocol to analyse alterations to muscle structure and function following eccentric exercise (Gleeson et al., 1995, Newham et al., 1983c, Schneider et al., 2007, Vissing et al., 2008).

The main findings of Chapter 3 demonstrated that damaged muscle has an increased reliance upon glucose oxidation to support contraction. This was inferred from a measureable increase in RER and calculated substrate oxidation. Since the one-legged cycling exercise in each leg was undertaken in adjacent 5 minute blocks and that oxygen consumption was the same, there can be some confidence that the blood delivery and thus circulating glucose availability to each leg was matched. Differences in the rate of whole body CHO utilisation must therefore reflect differential muscle glycogen oxidation. However, it is possible that EEIMD alters the process of contraction-induced glucose uptake by either increasing the reliance upon glycogen (and thus inhibiting glucose uptake) or increasing the capacity to take up and oxidise glucose rather than lipid, thereby producing a higher RER. Further, the supposition that the increased inflammatory response is creating an additional demand for substrate might explain the

increased reliance on CHO for the subsequent bout of exercise. Any additional glucose that is taken in by a damaged cell is quickly absorbed and utilised to assist in the muscle's repair and regeneration (Costill et al., 1990, Forster et al., 1989, Shearer et al., 1988). Given the localised and focal nature of damage to muscle cells, this may be less of a contributor to the reduction in whole muscle glucose uptake than has been proposed. The indirect (whole body) measures employed to assess substrate utilisation during exercise are not specific to the working leg and can only provide an insight to the substrate utilisation in a work matched model. Nevertheless, during exercise, particularly at moderate intensities and above, muscle metabolism overwhelmingly dictates whole body fuel selection. Another reason why there might be increased demand for glycogen by the damaged muscle may be if the cost of performing the eccentric exercise is to utilise the less oxidative motor units, i.e. the reverse 'Size Principle' (Nardone et al., 1989). This would mean the damaged muscle had used proportionally more glycogen to support its work, which will lead to reduced substrate supply for subsequent exercise. Capacity to function could be reduced by further depletion occurring in glycogen, which is an already under resourced store of intramuscular substrate. However, the time between testing bouts was probably long enough to ensure that this was not a significant factor.

Further research measuring arterio-venous difference and employing glucose tracers would be necessary to produce more definitive findings regarding the rate of glucose uptake during contraction and whether it is indeed impaired during exercise following EEIMD.

7.1.2 Whole body substrate storage

Pre-exercise concentrations of intramuscular substrates (muscle glycogen and triglyceride) largely dictates substrate selection during a bout of exercise (Coyle et al., 2001). Following the discussion of whole body substrate utilisation (section 7.1), mechanical damage conferred on the muscle as a result of prior eccentric exercise will alter muscle substrate utilisation during subsequent exercise. This assumption has been put forward as an explanation for an observed reduction in circulating TG concentration 4 days following an acute bout of eccentric exercise (Nikolaidis et al., 2008). According to these researchers, TG concentration may have been reduced because of an

increase in the activity of LPL in the capillary bed of damaged muscle, thereby allowing fatty acids to be taken up by skeletal muscle and either esterified in phospholipids or stored in the intramuscular TG 'pool'. Accordingly, the results of the subsequent experiment (Chapter 4) indicate that there was an effect of strenuous eccentric work on the ability to accrue IMCL in the damaged muscle during the 48 hours after exercise, with the greatest effect being evident at 24 hours. The cause of change in IMCL was outside the scope of Chapter 4. Nevertheless, changes in IMCL are likely a function of a change in the rate of FA uptake by the muscle or from a changed rate of utilisation.

Chapter 3 showed a greater reliance upon CHO during subsequent exercise, which suggests that the initially higher IMCL level in the damaged leg may have been a consequence of impaired intramuscular fatty acid oxidation rather than a greater FA uptake. Nevertheless, this explanation remains speculative especially since differing amounts of IMCL utilised during the concentric (control) contractions may have influenced the results. This because there is a larger metabolic cost for an equivalent amount of positive work due to tension being created by the contractile units only. In contrast, during eccentric exercise, the 'Size Principle' does not apply (Nardone et al., 1989) and the non-contractile proteins absorb some of the work. With the less oxidative motor units being recruited during the eccentric exercise, there may have been 'sparing' of IMCL utilisation. Any increase in LPL activity may be partly related to the increased demand by the working muscle for FA's, which can be utilised to replenish the muscle phospholipid layer and TG stores for the regeneration of damaged muscle fibres. In turn, this may have increased the FFA pool inside the muscle, increasing β-oxidation (for increased ATP generation) during any regeneration period of the muscle, which is evidenced in higher levels of resting energy expenditure in the days after eccentric exercise (Dolezal et al., 2000). A pre-exercise control IMCL measure was not undertaken because of limitation in resources. This would have provided insight to the relative reliance upon IMCL during eccentric and concentric contractions matched for work.

7.1.3 Insulin response and adaptation following EEIMD

Since changes in insulin sensitivity in muscle are associated with IMCL accretion (Goodpaster et al., 2001), any resulting changes in IMCL content as a result of EEIMD

could alter glycaemic control following acute bouts of eccentric exercise (Asp et al., 1996, King et al., 1993). It is well documented that multiple bouts of eccentric exercise attenuate markers of damage, such as structural integrity and force generating capacities of skeletal muscle during a subsequent eccentric exercise. Therefore, it is yet to be established whether metabolic alterations to eccentric exercise can be attenuated with eccentric exercise training. With a focus on further investigating 'transient insulin resistance' following EEIMD (Kirwan et al., 1992), the aim of Chapter 5 was to measure the glycaemic response to an oral glucose load after an acute bout and a repeat bout of eccentric exercise. At the time of data collection for this thesis only one study had been identified (Green et al., 2010) regarding adaptation and potential alterations in glycaemic control of skeletal muscle exposed to multiple bouts of eccentric exercise.

The results reported in Chapter 5 did not show any modification to the blood glucose or insulin response to an oral glucose load following either the acute bout or the repeat bout of eccentric exercise. In contrast, a recent study utilising a different eccentric exercise protocol (Green et al., 2010) showed an elevation in insulin and glucose responses after an acute bout of eccentrically biased exercise. This was attenuated with the repeat bout of eccentric exercise. Neither the current study nor that of Green et al. (2010) has revealed the mechanisms responsible for any alteration in the glycaemic responses to a repeated bout of eccentric contractions. A major underlying contributor to the proposed changes that would occur can be linked to the integrity of the cell membrane and its influence on the measures undertaken Chapter 5. However, potential membrane-related adaptations are important to consider when reduced glycogen synthesis (Costill et al., 1990, Doyle et al., 1993, O' Reilly et al., 1987, Widrick et al., 1992), 'transient insulin resistance' (King et al., 1993, Kirwan et al., 1991, Kirwan et al., 1992, Sherman et al., 1992) and decreased glucose transporter number (Asp et al., 1996) resulting from eccentric contractions are observed. These events closely relate and their normal function requires integrity of the cell membrane. Any adaptive alterations to the membrane after repeated eccentric contractions may reduce their severity.

Significant reductions in circulating CK measured following repeated bouts of eccentric exercise (Clarkson and Tremblay, 1988) may point to a possible mechanism for attenuation in damage. The trend towards a lower CK response after a repeated bout of

eccentric contractions observed in Chapter 5, suggests reduced cell-membrane damage to the same mechanical stress. Any enhancement to cell membrane integrity following a second bout of eccentric contractions could be accompanied by alterations to insulin receptor number, increased availability of glucose transporters and/or positive changes in the intracellular pathway for the disposal of glucose. Future studies are warranted to address the degree to which these factors play a role in the proposed mechanism. These may contribute to understanding the role of skeletal muscle in the dynamic state, which whole body insulin resistance can represent in healthy individuals.

In terms of showing changes to the glycaemic response following acute or repeat bouts of eccentric exercise, there were limitations in the design of Chapter 5. The methodology used to induce damage caused soreness and elevations in circulating CK but may not have been severe enough in any particular muscle to produce noticeable changes in whole body glycaemic control, or have damaged enough muscle to cause a marked impact on while body glucose control. To elucidate a suitable whole body response to OGTT the choice of squat based exercise was ecologically valid compared with a 'pure' eccentric model, such as the use of isokinetic dynamometry, which would only induce damage to a single group of muscles and therefore blunt the whole body response to the OGTT.

In line with previous studies, Chapter 5 observed the repeat bout effect when the same type of eccentric biased exercise was used for both the initial and the repeat bout of damage inducing exercise (Chen and Hsieh, 2001, Nosaka and Clarkson, 1995, Nosaka and Newton, 2002b). In practical terms, even trained muscles can experience EEIMD after performing unfamiliar movements and therefore, the magnitude of protective adaptation against subsequent eccentric exercise may decrease if the exercise involves a different motor unit recruitment pattern from the initial exercise. This same principal can be applied to the testing of muscle function and, more specifically, the ability to maximally generate force. Therefore, the use of the CMJ was justified as the movement mimicked the pattern of muscle recruitment during the squat exercise that was used as the eccentric loading protocol. This may highlight that there were alterations to the muscles involved in the movement due to maintenance of this performance measure in the repeat bout.

7.1.4 High energy phosphates

In Chapters 4 and 6, ^{31}P-MRS was used to evaluate changes in muscle metabolism at the cellular level at rest following acute and multiple bouts of eccentric exercise. The findings of Chapter 4 show a significant change in resting [P_i] and subsequently an increase in the P_i / PCr following EEIMD. However, this treatment had no effect on muscle pH, [PCr] or [ADP] and supports previous findings that ADP sensitivity is unchanged following eccentric exercise (Walsh et al., 2001).

Examining muscle at rest in Chapters 4 and 6, increased [P_i] resulted in an increase in the resting P_i / PCr ratio 24 and 48 hours after eccentric exercise. These findings are consistent with those of several previous studies using ^{31}P-MRS, which reported increases in resting P_i / PCr following eccentric exercise (Lund et al., 1998b, Lund et al., 1998a, McCully et al., 1992, Rodenburg et al., 1995). The elevated resting [P_i] may be interpreted as reflecting an increased rate of cellular metabolism (Lund et al., 1998b, Lund et al., 1998a, McCully et al., 1992, Rodenburg et al., 1995). Subsequently, this measure is not associated with the permeability of the membrane, as is the case with the serum CK measures (McCully et al., 1992). The repair and remodelling of tissue damaged via eccentric exercise could necessitate an increase in muscle metabolic rate (Yu et al., 2002, Yu and Thornell, 2002). 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) and may be a contributing factor in the change in substrate oxidation evident in Chapter 3. An alternative explanation for the increase in P_i /PCr, which importantly resulted from increased [P_i] but not decreased [PCr], may be linked to the breakdown of muscle tissue as a direct consequence of eccentric exercise. Disturbances in intracellular Ca^{2+} following eccentric exercise have been reported with associated activation of calcium-activated proteolytic pathways (Belcastro, 1993, Belcastro et al., 1998). Thus, the observed increase in resting [P_i] may not be due to increases in muscle metabolism but to degradation of muscle proteins and subsequent increase protein synthesis (McNeil and Khakee, 1992). Basic fibroblast growth factor (bFGF) is one of several growth factors that are biologically active in muscle growth control and its release in muscle could be directly coupled to plasma membrane damage

of muscle fibers. The release of bFGF has been shown to occur when cell membranes are disrupted (Muthukrishnan, Warder, and McNeil, 1991), such as those known to occur in eccentrically exercised muscle. Therefore, bFGF might be released through muscle fibre plasma membrane breaks. This mechanism could initiate growth in muscle damaged by exercise and thereby increase resting metabolism.

Increases in resting muscle [P_i] and P_i /PCr following periods of immobilisation have been implicated in loss of muscular strength (Pathare et al., 2005a, Pathare et al., 2005b). Increases in intracellular [P_i] 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 reported reduction in MVC following EEIMD may be related to the increased [P_i] and P_i /PCr. It could be speculated that reductions in force generating capacities are moderated by [P_i], however wide inter-subject variability was observed with the [P_i] value and thus it cannot be reliably concluded that [P_i] was a significant factor.

The findings of Chapter 6 show that muscle exposed to multiple bouts of eccentric exercise, which precede a larger bout of eccentric exercise, show similar P_i / PCr ratios as muscle previously exposed to only concentric exercise prior to the eccentric exercise. At the same time, measures of muscle function and CSA allow us to infer that the protocol utilised was sufficient to cause EEIMD. Therefore, whilst eccentric exercise training is well known to prevent DOMS, this is probably not associated with an attenuation of changes in intracellular metabolism. With no attenuation in the P_i / PCr ratio following exposure to multiple bouts of eccentric exercise and no significant drop in force generating capacity following the EEIMD, it can be argued that an increased [P_i] does not affect cross-bridge cycling as previously suggested. Rather a centrally mediated factor inhibiting force generation following an acute bout of eccentric exercise may be present and may be influential in attenuation of force generating capacity following multiple bouts of eccentric exercise.

It is apparent from both the findings of Chapters 4 and 6 that EEIMD does not have an impact on resting levels of PCr. Together, these results indicate that the concentrations of high energy phosphates, which are necessary for normal muscle cell function (Argov et al., 2000), are not affected by acute or chronically damaging bouts of eccentric

exercise. Further, the findings also indicate that neither eccentric nor concentric based bouts of training increased the resting [PCr]. This is in line with recent studies conducted on both endurance training and resistance training (Jones et al., 2007, Weber et al., 2010).

7.2 Conclusion

Studies presented in this thesis have examined indices of muscle damage and metabolism following EEIMD. Non-invasive methods of measuring muscle metabolism, indices of muscle injury as well as indirect biochemical indices of muscle injury, were used to examine function and adaptation of the muscle following eccentric exercise. Skeletal muscle has the ability to retain pools of short-term energy despite decrements in force generating capabilities and movement. This would indicate that peripheral factors within the muscle should be able to work, although centrally, there may be rate limiting steps in propagating the signal into myofibrils to allow contraction to take place. The results from this thesis have shown a number of main findings:

- Following acute bouts of eccentric exercise, subsequent concentric exercise bouts (24 and 48 hours later) are associated with a change in whole body substrate selection involving a higher reliance upon intramuscular CHO oxidation.
- Eccentric exercise resulting in EEIMD produces alterations to IMCL storage in comparison to a work matched control.
- High energy phosphate compounds are increased at rest in muscle exposed to acute bouts of eccentric contractions.
- Exposure to multiple bouts of squat based eccentric exercise does not confer a protective effect to measures of glycaemic response (insulin sensitivity and glucose disposal) in skeletal muscle, which has been exposed to a previous bout of the same eccentric exercise.
- When compared with regular concentric training, training muscle with an eccentric-bias does not prevent increases in resting high energy phosphate compounds in EEIMD.

7.2.1 Strengths & Limitations

During studies 1, 2 and 4 (Chapters 3, 4 and 6) a concurrent exercise model (single leg) was chosen. This single leg design allows for control of systemic factors such as diet and circulating cytokines. The use squat based exercise in study 3 (Chapter 5) may provide more ecologically valid model with which to study the effects of EEIMD on the whole body glycaemic control. It is believed the chosen exercise protocols were justified.

The use of a discrete observational period, usually 48 hours is chosen to observe changes in muscle function following eccentric exercise, this is to correspond with the period of maximal muscle soreness. However, the studies in this thesis attempted to evaluate time-course changes in the metabolic response 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 EEIMD such as the loss of force generating capacity. This influenced the choice of collecting MRS data at these time points rather than pre- and 48 hours post exercise as the access and cost to the MRS was a major factor in designing when to collect data.

The relatively small sample sizes used for the investigations that comprise this thesis may have increased the risk of type II statistical error being discovered. As limited research utilising MRS and EEIMD had been conducted it would be hard to conduct power calculations prior to the studies being done.

All participants were trained but unfamiliar to large amounts of eccentric exercise, and as such interpretation of the 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’ may last up to 6 months for most symptoms of EEIMD. Thus the training status of participants may have a profound influence on their metabolic response to EEIMD.

7.2.2 Implications & Future directions

The main findings of the studies that comprise this thesis have implications for the interpretation of whole body metabolic response to eccentric exercise. An awareness of potential changes in metabolic responses following eccentric exercise may help coaches, exercise scientists and health and fitness practitioners to make more informed decisions regarding advice given to any population. The increasing popularity of resistance and plyometric training methods may lead to an increased incidence of EEIMD in the active and sedentary population. Recreationally active individuals should be aware of the potential alterations to their performance and recovery capacity in the days following this type of training. Future research should also attempt to elucidate differences in the metabolic response to EEIMD between individuals of different training and activity status. A number of these findings could be translated to understanding the metabolic consequences in other instances of muscle damage such as external injury, changes with aging, muscular dystrophies and diabetes.

In future research, more work is needed to fully test the metabolic responses to EEIMD. Utilisation of non-invasive technology allows for determination of what is occurring in the muscle during various modalities of contraction. Whilst this thesis has extended the examination of some of these responses, many questions remain unanswered.

8. References

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9. Appendices

9.1 Study One

Participant characteristics

Participant	Age (years)	Height (cm)	Mass (kg)	VO_{2max} ($ml.kg.min^{-1}$)
MW	36	168	68	67.49
WC	33	182	71.5	54.32
JG	28	181	70.2	73.88
SH	29	183	70.7	68.43
MH	20	179	83	67.45
EM	31	178	78.6	61.23
PC	41	184	75.3	83.97
JH	30	181	84.3	62.75
Mean	**31.0**	**179.5**	**75.2**	**67.4**
SD	**6.1**	**5.0**	**6.1**	**8.9**

Participants one legged workloads

Participant	Warm Up (W)	Stage 1(W)	Stage 2(W)
MW	102	119	136
WC	71	88	106
JG	107	124	142
SH	107	124	142
MH	124	145	166
EM	77	96	116
PC	75	112	150
JH	85	106	127
Mean	**93.5**	**114.2**	**135.6**
SD	**19.1**	**17.9**	**19.1**

Participants creatine kinase activity ($IU.L^{-1}$)

Participant	Pre	24 hr	48 hr
MW	24	24	80
WC	100	349	403
JG	24	191	727
SH	24	144	271
MH	60	372	1510
EM	24	24	257
PC	36	145	462
JH	24	205	1070
Mean	**65**	**234**	**467**
SD	**32**	**101**	**334**

Participants isometric torque (Nm)

	Control			Damage		
Participant	**Pre**	**24 hr**	**48 hr**	**Pre**	**24 hr**	**48 hr**
MW	210	195	209	175	108	116
WC	204	159	201	147	99	137
JG	199	202	184	176	121	101
SH	252	260	235	245	273	298
MH	221	230	245	241	211	243
EM	255	248	227	266	238	228
PC	230	248	242	234	155	162
JH	257	245	251	251	186	142
Mean	**229**	**223**	**224**	**217**	**174**	**178**
SD	**24**	**35**	**24**	**44**	**64**	**70**

Participants concentric torque (Nm)

	Control			Damage		
Participant	**Pre**	**24 hr**	**48 hr**	**Pre**	**24 hr**	**48 hr**
MW	182	174	189	143	89	99
WC	146	122	143	145	91	95
JG	191	176	154	133	104	48
SH	168	202	181	175	194	212
MH	194	187	208	200	165	172
EM	234	221	201	209	201	176
PC	200	205	189	235	148	146
JH	241	215	238	245	175	144
Mean	**195**	**188**	**188**	**186**	**146**	**137**
SD	**32**	**32**	**30**	**43**	**46**	**53**

Participants eccentric torque (Nm)

	Control			Damage		
Participant	**Pre**	**24 hr**	**48 hr**	**Pre**	**24 hr**	**48 hr**
MW	111	226	238	153	127	133
WC	184	198	205	184	108	123
JG	235	213	190	189	142	91
SH	312	311	243	268	269	258
MH	286	287	299	260	244	249
EM	302	285	241	276	274	241
PC	268	262	259	286	188	197
JH	260	275	266	285	195	169
Mean	**245**	**257**	**243**	**238**	**193**	**183**
SD	**68**	**40**	**34**	**53**	**65**	**64**

Participant visual analogue scale concentric contraction (cm)

	Control			Damage		
Participant	**Pre**	**24 hr**	**48 hr**	**Pre**	**24 hr**	**48 hr**
MW	0	3.1	1.1	0	4.4	7.3
WC	0	0	0	0	3.2	8.3
JG	0	0	0	0	6.7	7.1
SH	0	0	1	0	3.1	2.2
MH	0	0	0	0	4.3	6.1
EM	0	2.2	6.3	0	5.2	8.7
PC	0	0	0	0	9.1	10
JH	0	0	0	0	5.2	8.1
Mean	**0**	**0.7**	**1.1**	**0**	**5.2**	**7.2**
SD	**0**	**1.3**	**2.2**	**0**	**2.0**	**2.3**

Participant visual analogue scale eccentric contraction (cm)

	Control			Damage		
Participant	**Pre**	**24 hr**	**48 hr**	**Pre**	**24 hr**	**48 hr**
MW	0	2.3	1.4	0	6.4	7.8
WC	0	0	0	0	3.5	8.9
JG	0	0	0	0	8.7	8.4
SH	0	1.1	2.1	0	3.6	2.6
MH	0	1.2	1.3	0	3.3	6.7
EM	0	0	4.1	0	5.4	5.6
PC	0	0	0	0	9.5	10
JH	0	1.2	0	0	6.6	8.8
Mean	**0**	**0.7**	**1.1**	**0**	**5.9**	**7.4**
SD	**0**	**0.9**	**1.5**	**0**	**2.4**	**2.4**

Participant respiratory exchange ratios (RER's)

Control

	Pre		24hr		48hr	
Participant	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**
MW	0.85	0.84	0.88	0.89	0.85	0.86
WC	0.91	0.92	0.89	0.91	0.90	0.90
JG	0.90	0.92	0.94	0.92	0.91	0.92
SH	0.90	0.91	0.91	0.91	0.87	0.89
MH	0.91	0.91	0.89	0.90	0.89	0.91
EM	0.88	0.90	0.90	0.89	0.94	0.91
PC	0.82	0.86	0.85	0.85	0.85	0.87
JH	0.97	0.96	0.89	0.89	0.95	0.95
Mean	**0.89**	**0.90**	**0.89**	**0.89**	**0.90**	**0.90**
SD	**0.04**	**0.04**	**0.02**	**0.02**	**0.04**	**0.03**

Damage

	Pre		24hr		48hr	
Participant	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**
MW	0.84	0.84	0.89	0.93	0.88	0.91
WC	0.95	0.95	0.96	1.00	0.97	0.93
JG	0.90	0.90	0.93	0.96	0.94	0.93
SH	0.94	0.93	0.92	0.91	0.91	0.91
MH	0.92	0.91	0.92	0.92	0.92	0.93
EM	0.89	0.88	0.89	0.89	0.93	0.91
PC	0.84	0.86	0.90	0.91	0.91	0.93
JH	0.95	0.95	0.94	0.97	0.96	0.97
Mean	**0.90**	**0.90**	**0.92**	**0.94**	**0.93**	**0.93**
SD	**0.04**	**0.04**	**0.03**	**0.04**	**0.03**	**0.02**

Estimated substrate utilisation during one legged cycling Control (CHO) (g/min)

	Pre		24hr		48hr	
Participant	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**
MW	1.52	1.59	1.87	2.14	1.11	1.31
WC	1.99	2.31	1.78	2.17	1.86	2.03
JG	2.21	2.55	2.38	2.53	1.91	2.47
SH	1.88	2.39	2.15	2.19	1.94	2.34
MH	2.64	3.19	2.64	3.00	3.16	3.14
EM	2.23	2.39	1.95	2.42	1.87	2.39
PC	1.01	1.61	1.09	1.46	1.15	1.66
JH	2.61	2.92	1.87	2.15	2.47	2.78
Mean	**2.01**	**2.37**	**1.97**	**2.26**	**1.93**	**2.27**
SD	**0.55**	**0.56**	**0.46**	**0.44**	**0.66**	**0.59**

Damage (CHO) (g/min)

	Pre		24hr		48hr	
Participant	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**
MW	1.44	1.69	2.29	3.16	1.39	1.82
WC	2.05	2.51	2.53	3.63	2.58	2.53
JG	2.61	2.92	2.52	2.76	2.32	2.57
SH	1.88	2.02	2.15	2.83	2.37	2.55
MH	2.75	3.09	2.66	3.09	3.17	3.38
EM	2.10	2.22	1.37	2.39	2.15	2.60
PC	1.11	1.62	1.51	2.02	1.54	2.28
JH	2.56	2.84	2.56	3.74	2.39	3.52
Mean	**2.06**	**2.36**	**2.20**	**2.95**	**2.24**	**2.66**
SD	**0.58**	**0.57**	**0.50**	**0.58**	**0.57**	**0.55**

Control (Lipid) (g/min)

	Pre		24hr		48hr	
Participant	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**
MW	0.58	0.72	0.46	0.49	0.42	0.42
WC	0.30	0.29	0.36	0.35	0.36	0.37
JG	0.41	0.41	0.38	0.41	0.53	0.52
SH	0.35	0.32	0.21	0.32	0.30	0.30
MH	0.65	0.61	0.51	0.63	0.33	0.48
EM	0.34	0.39	0.41	0.43	0.44	0.39
PC	0.54	0.51	0.41	0.55	0.43	0.48
JH	0.11	0.19	0.39	0.49	0.18	0.19
Mean	**0.41**	**0.43**	**0.39**	**0.46**	**0.37**	**0.39**
SD	**0.17**	**0.18**	**0.09**	**0.10**	**0.11**	**0.11**

Damage (Lipid) (g/min)

	Pre		24hr		48hr	
Participant	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**
MW	0.64	0.73	0.50	0.36	0.35	0.30
WC	0.16	0.17	0.13	0.00	0.10	0.30
JG	0.27	0.33	0.35	0.43	0.39	0.44
SH	0.35	0.41	0.23	0.18	0.24	0.28
MH	0.64	0.75	0.59	0.70	0.39	0.52
EM	0.29	0.37	0.19	0.34	0.29	0.29
PC	0.46	0.51	0.30	0.34	0.27	0.27
JH	0.18	0.21	0.23	0.14	0.13	0.16
Mean	**0.37**	**0.43**	**0.32**	**0.31**	**0.27**	**0.32**
SD	**0.19**	**0.22**	**0.16**	**0.21**	**0.11**	**0.11**

9.2 Study Two

Participant Characteristics

Participant	Age (years)	Height (cm)	Mass (kg)	Body Fat (%)	VO_{2max} ($ml.kg.min^{-1}$)
JG	26	181	70.6	11	73.9
JH	27	181	90.4	21	58.8
NJ	31	175	66.3	14.8	66.2
TM	28	166	62.3	9	68.7
PC	40	184	70.5	11	83.9
SS	39	172	74.1	11	76.5
Mean	**32**	**176**	**72.4**	**12.9**	**71.3**
SD	**6**	**7**	**9.7**	**4.4**	**8.8**

IMCL / Cr from JMRUI

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
JG	8.6	21.8	8.1	10.8
JH	13.0	23.9	36.1	36.1
NJ	6.0	8.1	8.0	5.3
TM	6.5	13.3	6.1	3.0
PC	5.8	4.6	4.0	6.5
SS	4.2	4.1	5.9	5.8
Mean	**7.2**	**12.1**	**10.5**	**10.6**
SD	**2.9**	**7.9**	**11.4**	**11.5**

Absolute IMCL (mmol/kg/w.w)

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
JG	15.4	20.1	11.2	12.7
JH	12.6	18.2	27.5	29.9
NJ	12.8	15.3	12.4	13.9
TM	7.8	10.8	10.1	6.8
PC	10.7	9.8	6.7	10
SS	10.9	8.1	8.7	7.1
Mean	**12.3**	**14.5**	**11.6**	**12.6**
SD	**2.8**	**4.9**	**7.4**	**8.1**

Resting [PCr] (mM)

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
JG	22.47	18.08	20.58	29.96
JH	33.05	28.77	33.42	29.59
NJ	27.27	21.52	24.47	21.85
TM	22.24	20.96	20.70	31.87
PC	23.50	23.50	25.98	28.14
SS	31.45	28.18	27.53	32.06
Mean	**26.66**	**23.50**	**25.45**	**28.91**
SD	**4.72**	**4.23**	**4.80**	**3.76**

Resting [P_i] (mM)

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
JG	3.20	2.38	3.66	5.23
JH	5.25	4.21	7.69	7.53
NJ	4.23	2.96	4.30	4.26
TM	3.42	2.93	4.79	6.48
PC	4.39	5.45	6.45	6.90
SS	3.85	3.53	5.57	6.19
Mean	**4.06**	**3.58**	**5.41**	**6.10**
SD	**0.74**	**1.11**	**1.48**	**1.18**

Resting P_i / PCr

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
JG	0.14	0.13	0.18	0.17
JH	0.16	0.15	0.23	0.25
NJ	0.16	0.14	0.18	0.20
TM	0.15	0.14	0.23	0.20
PC	0.19	0.23	0.25	0.25
SS	0.12	0.13	0.20	0.19
Mean	**0.15**	**0.15**	**0.21**	**0.21**
SD	**0.02**	**0.04**	**0.03**	**0.03**

Resting [ADP] (µM)

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
JG	43.06	43.72	43.98	43.52
JH	40.77	41.87	39.02	41.22
NJ	44.33	44.45	41.39	45.20
TM	43.61	44.97	40.24	41.02
PC	45.82	45.82	49.21	49.16
SS	42.94	41.62	45.64	44.24
Mean	**43.42**	**43.74**	**43.25**	**44.06**
SD	**1.67**	**1.69**	**3.80**	**3.00**

Resting pH

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
JG	7.01	7.01	7.02	7.01
JH	6.99	7.00	6.97	6.99
NJ	7.02	7.02	6.99	7.03
TM	7.01	7.03	6.98	6.99
PC	7.04	7.04	7.07	7.07
SS	7.01	6.99	7.03	7.02
Mean	**7.01**	**7.02**	**7.01**	**7.02**
SD	**0.02**	**0.02**	**0.04**	**0.03**

Quadriceps muscle volume (cm^3)

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
JG	1124.67	1155.71	1268.31	1176.38
JH	1331.80	1314.96	1557.66	1525.86
NJ	938.66	870.73	943.66	905.94
TM	898.00	928.80	1130.22	1067.19
PC	1312.71	1312.95	1368.31	1310.20
SS	1114.08		1297.53	
Mean	**1119.99**	**1116.63**	**1260.95**	**1197.11**
SD	**181.23**	**209.26**	**209.15**	**236.09**

9.3 Study Three

Participant characteristics

Participant	Age (years)	Height (cm)	Mass (kg)	Body Fat (%)
BW	41	181	70.4	15.5
GM	27	182	70.1	21
MW	35	170	69.5	14.5
WC	32	181	71.5	17.5
JE	20	180	88	20
DS	35	179	84	20
BS	23	173	85.4	17
PP	38	179	93.5	14
Mean	**31.4**	**178**	**79**	**17.4**
SD	**7.4**	**4.6**	**9.7**	**2.7**

Participants isometric torque (Nm)

	SE1				SE2			
Participant	**Pre**	**24 hr**	**48 hr**	**96 hr**	**Pre**	**24 hr**	**48 hr**	**96hr**
BW	536.1	423.2	453.4	502.7	549.1	480.1	495.2	527.4
GM	338.9	251.1	206.7	348.5	347.7	327.4	293.4	267.6
MW	474.1	496.1	508.2	514.4	486.2	463.9	453.5	517.1
WC	528.1	397.4	341.6	421.3	557.2	484.2	461.8	488.9
JE	767.1	724.1	829.9	814.1	774.2	702.3	710.7	771.6
DS	716.1	439.3	495.5	531.1	742.1	605.4	512.3	521.7
BS	1071.1	885.2	834.3	892.6	1091.1	912.2	911.7	896.4
PP	533.4	644.6	671.1	689.8	539.5	634.8	685.1	747.5
Mean	**620.6**	**516.6**	**524.2**	**574.9**	**635.9**	**567.9**	**548.4**	**570.1**
SD	**226.1**	**204.6**	**223.1**	**190.9**	**228.6**	**179.4**	**192.8**	**199.8**

Participants countermovement jump (cm)

	SE1				SE2			
Participant	**Pre**	**24 hr**	**48 hr**	**96 hr**	**Pre**	**24 hr**	**48 hr**	**96hr**
BW	32.4	32.9	36.5	37.1	32.3	32.7	35.3	34.0
GM	40.9	30.5	27.3	29.9	41.5	32.5	33.3	32.2
MW	37.1	34.9	34.7	34.9	37.2	34.3	36.4	36.1
WC	46.2	38.0	35.2	41.2	45.7	41.3	38.3	40.8
JE	45.0	37.2	38.9	41.5	44.0	40.4	44.4	39.5
DS	39.2	37.5	42.4	41.6	39.6	46.6	45.8	47.1
BS	41.9	43.8	44.7	44.4	40.9	44.9	44.3	44.8
PP	36.9	37.8	36.2	38.0	37.9	39.1	35.6	39.0
Mean	**39.9**	**36.6**	**37.0**	**38.6**	**39.9**	**39.0**	**39.2**	**39.2**
SD	**4.6**	**4.0**	**5.3**	**4.6**	**4.2**	**5.4**	**4.9**	**5.1**

Participant visual analogue scale (cm)

	SE1				SE2			
Participant	**Pre**	**24 hr**	**48 hr**	**96 hr**	**Pre**	**24 hr**	**48 hr**	**96hr**
BW	0	4.3	6.7	4.4	0	3.5	4.5	2.7
GM	0	5.4	7.3	5.6	0	4.2	4.8	3.4
MW	0	6.3	7.5	6.0	0	4.5	5.3	2.6
WC	0	6.8	7.8	6.3	0	4.6	5	3.4
JE	0	4.7	6.7	4.6	0	3.4	4	2.7
DS	0	5.1	6.7	5.0	0	4.5	4.9	3.4
BS	0	5	6.6	4.5	0	3.8	4.4	2.9
PP	0	5.2	7.2	3.6	0	3.5	4.9	1.8
Mean	**0**	**5.4**	**7.1**	**5**	**0**	**4**	**4.7**	**2.9**
SD	**0**	**0.8**	**0.5**	**0.9**	**0**	**0.5**	**0.4**	**0.6**

Area under the curve

	Glucose			Insulin		
Participant	**Control**	**SE1**	**SE2**	**Control**	**SE1**	**SE2**
BW	600.8	721.5	596.3	53166.8	41223.0	45742.5
GM	633.0	645.8	572.3	28722.8	20211.0	19041.0
MW	851.3	752.3	723.8	24044.3	25623.8	30163.5
WC	686.3	611.3	687.0	42246.8	52924.5	56788.5
JE	923.3	939.8	1071.8	87365.3	96863.3	70089.0
DS	696.8	642.0	671.3	51304.5	42872.3	54294.8
BS	618.8	574.5	769.5	63361.5	54672.0	36960.0
PP	709.5	716.3	599.3	52562.3	84300.8	67817.3
Mean	**714.9**	**700.4**	**711.4**	**50346.8**	**52336.3**	**47612.1**
SD	**114.7**	**113.9**	**160.7**	**19923.3**	**26642.7**	**18047.7**

Participants creatine kinase activity ($IU.L^{-1}$)

		Control			SE1			SE2		
Participant	**Pre**	**24 hr**	**48 hr**	**96 hr**	**24 hr**	**48 hr**	**96 hr**	**24 hr**	**48 hr**	**96hr**
BW	80	81	89	67	328	194	98	86	81	62
GM	135	186	144	137	173	127	107	171	151	135
MW	218	221	239	160	393	282	172	229	186	140
WC	77	94	151	108	367	187	128	146	110	88
JE	79	99	191	170	210	145	120	376	236	187
DS	71	87	92	101	1322	962	325	195	119	77
BS	222	96	81	355	205	287	104	126	98	288
PP	78	104	105	81	268	133	84	216	135	103
Mean	**120**	**121**	**136**	**147**	**408**	**289**	**142**	**193**	**139**	**135**
SD	**65**	**52**	**56**	**91**	**378**	**279**	**78**	**88**	**51**	**74**

Participants fasting glucose (mmol/L)

				Control				SE1				SE2		
Participant	**Pre**	**24 hr**	**48 hr**	**96 hr**	**Pre**	**24 hr**	**48 hr**	**96 hr**	**Pre**	**24 hr**	**48 hr**	**96hr**
BW	4.9	5.1	5.1	6.8	4.9	5.2	5.2	6.5	4.9	5.0	5.2	6.6
GM	5.1	4.7	5.0	7.2	5.1	4.8	5.0	6.6	5.1	4.8	5.0	6.0
MW	5.0	5.0	5.6	6.5	5.0	5.1	5.0	5.6	5.0	5.4	5.9	6.2
WC	5.7	5.6	5.3	7.7	5.7	5.1	5.1	6.2	5.7	5.2	5.2	6.9
JE	6.0	4.7	4.9	6.6	6.0	4.8	4.7	7.2	6.0	4.8	5.1	6.1
DS	5.3	5.1	5.2	5.9	5.3	5.3	4.8	5.5	5.3	5.0	5.2	5.9
BS	4.5	4.9	4.5	5.3	4.5	4.3	4.0	6.9	4.5	4.8	5.5	7.8
PP	5.8	6.0	5.7	6.7	5.8	5.5	5.7	8.1	5.8	5.6	4.6	5.7
Mean	**5.3**	**5.1**	**5.2**	**6.6**	**5.3**	**5.0**	**4.9**	**6.6**	**5.3**	**5.1**	**5.2**	**6.4**
SD	**0.5**	**0.4**	**0.4**	**0.7**	**0.5**	**0.4**	**0.5**	**0.9**	**0.5**	**0.3**	**0.4**	**0.7**

OGTT glucose response (mmol/L)

Time in minutes

	Control							SE1							SE2						
Participant	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**0**	**15**	**30**	**45**	**60**	**90**	**120**
BW	5.1	6.8	6.9	6.5	4.6	3.6	3.2	5.2	6.5	8.4	7.0	6.6	4.4	4.9	5.2	6.6	7.1	5.1	4.3	4.3	3.3
GM	5.0	7.2	7.2	6.0	4.8	4.2	3.7	5.0	6.6	8.0	6.6	5.3	4.1	3.8	5.0	6.0	6.1	5.0	4.5	3.9	4.0
MW	5.6	6.5	7.7	7.8	7.5	6.5	7.7	5.0	5.6	7.0	7.3	6.5	6.1	5.8	5.9	6.2	7.1	7.9	7.0	5.0	3.6
WC	5.3	7.7	8.9	5.8	5.6	4.6	3.1	5.1	6.2	7.4	6.6	4.0	4.3	3.4	5.2	6.9	7.8	8.1	5.8	4.5	2.7
JE	4.9	6.6	10.2	9.6	8.8	7.1	5.3	4.7	7.2	8.7	9.7	9.2	7.5	5.9	5.1	6.1	9.9	11.2	11.0	10.3	4.6
DS	5.2	5.9	6.6	6.8	6.5	5.2	4.4	4.8	5.5	7.0	7.4	6.2	4.0	3.2	5.2	5.9	6.8	7.8	7.5	3.5	3.4
BS	4.5	5.3	7.8	6.7	5.8	4.2	2.1	4.0	6.9	6.4	5.4	4.8	4.0	2.4	5.5	7.8	9.5	6.7	5.7	6.1	3.8
PP	5.7	6.7	8.8	6.9	5.9	4.8	3.6	5.7	8.1	8.8	6.2	5.4	4.7	4.3	4.6	5.7	9.1	6.6	4.3	3.2	3.4
Mean	**5.2**	**6.6**	**8.0**	**7.0**	**6.2**	**5.0**	**4.1**	**4.9**	**6.6**	**7.7**	**7.0**	**6.0**	**4.9**	**4.2**	**5.2**	**6.4**	**7.9**	**7.3**	**6.3**	**5.1**	**3.6**
SD	**0.4**	**0.7**	**1.2**	**1.2**	**1.4**	**1.2**	**1.7**	**0.5**	**0.9**	**0.9**	**1.3**	**1.6**	**1.3**	**1.2**	**0.4**	**0.7**	**1.4**	**2.0**	**2.3**	**2.3**	**0.6**

OGTT insulin response (pmol/L)

Time in minutes

	Control							SE1							SE2						
Participant	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**0**	**15**	**30**	**45**	**60**	**90**	**120**
BW	13.4	58.6	82.5	120.6	77.5	50.6	25.6	10.0	33.0	104.1	64.8	61.8	34.7	27.8	3.5	50.8	104.8	56.9	63.7	54.1	22.3
GM	15.9	59.0	52.5	47.1	34.2	22.7	13.7	8.3	30.4	40.2	29.5	26.5	20.2	10.7	10.7	34.5	37.8	33.6	24.7	11.1	13.3
MW	4.4	10.0	38.7	38.3	27.9	28.7	43.6	7.4	17.2	45.6	41.5	38.3	28.3	25.0	8.5	18.9	56.0	81.1	35.2	30.5	16.7
WC	8.9	56.9	132.8	47.4	67.4	27.1	9.7	8.6	50.4	110.8	152.6	36.4	59.5	17.5	6.9	49.2	64.2	146.0	89.7	66.5	15.9
JE	16.5	60.5	105.6	104.1	172.6	122.9	56.7	19.7	83.5	114.8	158.1	148.0	133.2	76.2	15.0	41.9	88.2	114.7	93.6	121.4	38.4
DS	11.8	54.4	58.7	82.4	78.3	72.7	29.2	7.8	24.8	58.5	69.9	88.3	47.2	28.7	16.1	29.8	79.5	111.2	103.3	45.9	46.8
BS	10.4	40.0	139.2	159.8	128.5	31.5	9.5	9.6	115.8	173.5	73.5	53.6	34.3	9.3	9.2	50.5	118.4	75.0	23.9	26.6	18.5
PP	25.6	48.3	164.5	98.4	65.0	33.1	18.1	25.7	128.7	262.7	114.1	76.3	65.4	46.9	27.2	96.7	134.6	153.9	106.4	39.8	14.1
Mean	**13.4**	**48.5**	**96.8**	**87.3**	**81.4**	**48.7**	**25.8**	**12.1**	**60.5**	**113.8**	**88.0**	**66.2**	**52.9**	**30.3**	**12.1**	**46.5**	**85.4**	**96.5**	**67.6**	**49.5**	**23.3**
SD	**6.3**	**17.0**	**45.9**	**42.1**	**48.0**	**34.1**	**17.0**	**6.8**	**43.3**	**74.6**	**48.5**	**39.1**	**35.9**	**22.1**	**7.3**	**23.2**	**32.7**	**42.3**	**35.4**	**33.7**	**12.5**

Participants fasting insulin (pmol/L)

	Control				SE1				SE2			
Participant	**Pre**	**24 hr**	**48 hr**	**96 hr**	**Pre**	**24 hr**	**48 hr**	**96 hr**	**Pre**	**24 hr**	**48 hr**	**96hr**
BW	9.9	10.0	13.4	58.6	9.9	12.1	10.0	33.0	9.9	4.7	3.5	50.8
GM	15.8	13.1	15.9	59.0	15.8	8.2	8.3	30.4	15.8	6.8	10.7	34.5
MW	4.8	4.8	4.4	10.0	4.8	7.4	7.4	17.2	4.8	12.7	8.5	18.9
WC	6.6	11.6	8.9	56.9	6.6	13.5	8.6	50.4	6.6	7.8	6.9	49.2
JE	15.2	20.8	16.5	60.5	16.9	20.8	19.7	83.5	15.8	24.0	15.0	41.9
DS	14.0	11.8	11.8	54.4	14.0	9.8	7.8	24.8	14.0	11.1	16.1	29.8
BS	10.9	11.3	10.4	40.0	10.9	10.5	9.6	115.8	10.9	15.4	9.2	50.5
PP	16.9	18.0	25.6	48.3	16.9	24.6	25.7	128.7	16.9	24.0	27.2	96.7
Mean	**11.8**	**12.7**	**13.4**	**48.5**	**12.0**	**13.4**	**12.1**	**60.5**	**11.8**	**13.3**	**12.1**	**46.5**
SD	**4.5**	**4.9**	**6.3**	**17.0**	**4.7**	**6.2**	**6.8**	**43.3**	**4.5**	**7.4**	**7.3**	**23.2**

9.4 Study Four

Participant Characteristics

Participant	Age (years)	Height (cm)	Mass (kg)	Body Fat (%)	VO_{2max} ($ml.kg.min^{-1}$)
SS	40	172	74.4	11	75.4
JH	29	181	82.3	17	63.4
AH	45	168.5	63.9	24	55.6
NJ	31	175	66.3	14.8	66.2
PC	40	184.5	73.5	11	83.9
TM	28	166	65.4	9	68.7
Mean	**35.5**	**174.5**	**70.9**	**14.5**	**68.9**
SD	**7.1**	**7.2**	**7.1**	**5.5**	**9.8**

Participants Isometric torque (Nm)

	ConTr			EccTr		
Participant	**Pre Train**	**Post Train**	**Post Damage**	**Pre Train**	**Post Train**	**Post Damage**
SS	294.0	307.4	204.4	339.2	350.0	213.9
JH	248.1	271.3	210.6	205.4	247.7	246.2
AH	163.7	200.2	160.4	169.8	185.5	163.8
NJ	231	265	197	235	276	228
PC	236.5	318.0	221.3	249.9	347.0	333.0
TM	212.0	227.8	189.4	210.0	250.1	182.5
Mean	**231**	**265**	**197**	**235**	**276**	**228**
SD	**43**	**45**	**21**	**58**	**64**	**60**

Participants concentric torque (Nm)

	ConTr			EccTr		
Participant	**Pre Train**	**Post Train**	**Post Damage**	**Pre Train**	**Post Train**	**Post Damage**
SS	239.0	313.9	211.3	309.0	276.2	206.3
JH	235.9	336.0	304.9	225.2	336.5	324.3
AH	148.2	173.5	136.8	203.3	169.5	163.3
NJ	211	261.0	226	237	249	239
PC	268.0	281.0	300.0	248.4	267.2	259.0
TM	165.0	198.2	176.7	197.0	195.4	243.8
Mean	**211**	**261**	**226**	**237**	**249**	**239**
SD	**46**	**64**	**67**	**40**	**60**	**54**

Participants eccentric torque (Nm)

	ConTr			EccTr		
Participant	**Pre Train**	**Post Train**	**Post Damage**	**Pre Train**	**Post Train**	**Post Damage**
SS	309.0	358.8	218.6	334.0	381.0	360.3
JH	253.9	397.1	352.1	294.5	391.0	360.0
AH	223.4	233.2	167.7	237.2	246.7	205.3
NJ	241	322	254	264	334	336
PC	311.5	366.0	303.4	333.5	388.0	368.0
TM	109.0	256.7	228.2	123.0	262.7	385.3
Mean	**241**	**322**	**254**	**264**	**334**	**336**
SD	**74**	**65**	**65**	**79**	**65**	**66**

Resting [PCr] (mM)

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
SS	20.92	15.89	15.77	18.95
JH	15.86	19.77	19.64	20.61
AH	19.21	16.22	15.90	17.90
NJ	28.14	22.27	21.04	23.97
PC	14.79	24.19	19.23	15.21
TM	16.91	17.88	17.92	19.97
Mean	**19.30**	**19.37**	**18.25**	**19.44**
SD	**4.87**	**3.35**	**2.12**	**2.92**

Resting [P_i] (mM)

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
SS	4.21	3.16	3.75	3.95
JH	3.49	4.39	4.04	3.64
AH	2.80	2.14	2.55	2.65
NJ	5.89	5.12	3.81	4.43
PC	3.93	5.05	4.04	3.58
TM	3.87	4.35	3.77	4.12
Mean	**4.03**	**4.03**	**3.66**	**3.73**
SD	**1.03**	**1.17**	**0.56**	**0.62**

Resting P_i / PCr

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
SS	0.21	0.15	0.24	0.21
JH	0.18	0.22	0.21	0.18
AH	0.15	0.15	0.16	0.15
NJ	0.18	0.21	0.18	0.18
PC	0.24	0.27	0.21	0.24
TM	0.21	0.23	0.21	0.21
Mean	**0.21**	**0.21**	**0.20**	**0.19**
SD	**0.04**	**0.04**	**0.03**	**0.03**

Resting [ADP] (µM)

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
SS	41.47	47.36	46.80	46.77
JH	38.67	39.74	39.83	36.59
AH	38.71	37.09	41.49	39.09
NJ	45.31	41.91	44.30	42.45
PC	48.50	44.16	46.31	48.29
TM	43.68	49.78	41.25	41.99
Mean	**42.72**	**43.34**	**43.33**	**42.53**
SD	**3.87**	**4.74**	**2.89**	**4.44**

Resting pH

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
SS	6.99	7.05	7.04	7.05
JH	6.96	6.97	6.98	6.94
AH	6.96	6.94	6.99	6.97
NJ	7.03	7.00	7.02	7.00
PC	7.06	7.02	7.04	7.06
TM	7.01	7.07	6.99	7.00
Mean	**7.00**	**7.01**	**7.01**	**7.00**
SD	**0.04**	**0.05**	**0.03**	**0.05**

Quadriceps muscle volume (cm3)

	Control		Damage	
Participant	**24 hr**	**48 hr**	**24 hr**	**48 hr**
SS	1264	1346	1338	1314
JH	1377	1425	1281	1314
AH	762	867	779	783
NJ	918	938	887	870
PC	1406	1398	1279	1312
TM	1006	1130	1061	997
Mean	**1122**	**1184**	**1104**	**1098**
SD	**265**	**243**	**233**	**245**

9.5 Publication

Eur J Appl Physiol (2010) 110:1135–1141
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ORIGINAL ARTICLE

Effects of eccentric exercise-induced muscle damage on intramyocellular lipid concentration and high energy phosphates

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Abstract Eccentric exercise is known to cause changes to the ultrastructure of skeletal muscle and, in turn, may alter the ability of the muscle to store and utilise intracellular substrates such as intramyocellular lipid (IMCL). The purpose of this study was to test the hypothesis that exercise-induced muscle damage (EIMD) results in IMCL accumulation. Six males (31 ± 6 years; mean ± SD, and 72.3 ± 9.7 kg body mass) performed 300 unilateral, maximal, isokinetic, eccentric contractions (Ecc) (30° s^{-1}) of the quadriceps on an isokinetic dynamometer, followed immediately by an equal amount of work by the contralateral leg but with concentric action (Con). Phosphate compounds and IMCL content of the *vastus lateralis* of both legs were measured using ^{31}P and ^{1}H magnetic resonance spectroscopy. IMCL content was higher in Ecc than Con 24 h post but the reverse was evident 48 h post-exercise ($P = 0.046$). A significant time × trial interaction for resting [P_i] ($P = 0.045$), showed increases in Ecc across time but no change in Con. A significant main effect of trial ($P = 0.002$) was apparent indicating the Ecc leg had marked metabolic dysfunction. The P_i/PCr ratio showed a significant effect of trial ($P = 0.001$) with an increase evident in Ecc leg, primarily due to increases in [P_i]. The present study highlights changes in IMCL content of skeletal muscle following EIMD.

Keywords Eccentric exercise · Intramyocellular lipid · Inorganic phosphate · Muscle damage · Magnetic resonance spectroscopy

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Introduction

Exercise-induced muscle damage (EIMD) occurs following eccentric work to which the muscle is unaccustomed. The symptoms of EIMD are well defined and characterised by disruption to normal sarcomere arrangement of a muscle fibre (Newham et al. 1983) cytoskeletal and membrane disruption (Feasson et al. 2002; Hamer et al. 2002; Lovering and De Deyne 2004), loss of calcium homeostasis (Balnave et al. 1997), excitation–contraction coupling impairment (Warren et al. 1993), and loss of force production (Byrne et al. 2004).

Although muscle contraction improves sensitivity of skeletal muscle to insulin-mediated glucose uptake in the short and longer term (Holloszy et al. 1998), paradoxically, strenuous eccentric muscular work has been shown to reduce resting muscle insulin sensitivity (Del Aguila et al. 2000). Although there is no definitive explanation for this impairment, it has been proposed that the reduced insulin sensitivity results from a systemic inflammatory response to EIMD (Costill et al. 1990).

Whole body insulin resistance in obesity, lipid infusion and starvation is closely associated with accumulation and

9.6 Statement of Contribution

DRC 16

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STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: JONATHAN HUGHES

Name/Title of Principal Supervisor: A/PROF STEVE STANNARD

Name of Published Research Output and full reference: EFFECTS OF ECCENTRIC EXERCISE - INDUCED MUSCLE DAMAGE ON INTRAMYOCELLULAR LIPID CONCENTRATION AND HIGH ENERGY PHOSPHATES. EUROPEAN JOURNAL OF APPLIED PHYSIOLOGY, 110(6), 1135

In which Chapter is the Published Work: 4

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: 80%

  and / or

- Describe the contribution that the candidate has made to the Published Work:

Candidate's Signature — Date: 21/11/11

Principal Supervisor's signature — Date: 21/11/11

GRS Version 3– 16 September 2011