Indirect measures of substrate utilisation following exercise induced muscle damage

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Abstract

This study investigated whether exercise-induced muscle damage (EIMD) resulted in changes to whole-body substrate utilisation during exercise performed during the subsequent 48 hours. Eight males (31±6 years) performed 30 minutes of bench stepping exercise. 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 benchstepping exercise 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 force, 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=0.02). A significant treatment × time × effort interaction (P<0.01) 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. Suggesting that in EIMD, reductions in strength and increased feelings of soreness can be associated with greater reliance upon intramuscular CHO oxidation, than lipid, during subsequent concentric work.

Keywords: Eccentric exercise, metabolism, respiratory exchange ratio

Introduction

When compared with concentric activity of similar intensity, strenuous eccentric work is associated with selective damage to the fast twitch fibres (Fridén, Sjostrom, & Ekblom, 1983; Jones, Newham, Round, & Tolfree, 1986) and more rapidly depletes fast-twitch fibres of glycogen (Fridén, Seger, Sjostrom, & Ekblom, 1983; Hickner et al., 1997) than slow-twitch fibres (O' Reilly et al., 1987).

Following eccentric exercise, glucose uptake by damaged skeletal muscle fibres is reduced compared with muscle that has undertaken concentric exercise, and considered undamaged (Costill et al., 1990; Doyle, Sherman, & Strauss, 1993; O' Reilly et al., 1987; Widrick et al., 1992). Furthermore, it has been demonstrated that glycogen resynthesis is impaired following EIMD (Asp, Daugaard, Kristiansen, Kiens, & Richter, 1998; Asp, Daugaard, & Richter, 1995; Costill et al., 1990; Widrick et al., 1992). Pre-exercise availability of intramuscular glycogen and triglycerides largely dictates fuel selection during exercise (Coyle, Jeukendrup, Oseto, Hodgkinson, & Zderic, 2001; Johnson et al., 2003). 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 (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 EIMD 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 EIMD produces changes to the substrate utilisation of skeletal muscle that is undertaking a subsequent bout of concentric exercise. And more specifically, 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 EIMD condition. To test the hypothesis, 8 men performed eccentric exercise with 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.

Methodology

Participants

Eight males (mean±**SD**; age 31±6 years; height, 179±5 cm; $\dot{V}O_{2max}$, 67.4±8.9 mL·kg^{-1}·min^{-1} and body mass 75±6 kg) who 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 baseline measures required the subjects to pedal the cycle ergometer for 15 min firstly with the right leg, then the left during which time metabolic measurements were made. During this visit, measures of muscle function were also made. After a short period of rest, subjects then undertook a 30min bout of bench stepping exercise with an additional 10kg mass. The fifth and sixth sessions were repeats of the baseline cycling and muscle function sessions.

The study was conducted in a randomised balanced design. That is, 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 first, followed by the left leg. On each experimental visit to the laboratory participants reported after an overnight fast. Measures of muscular performance (isometric force, concentric and eccentric torque) and capillary blood creatine kinase (CK) activity were made. Perceived muscular soreness was recorded, and during the cycling exercise, 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.

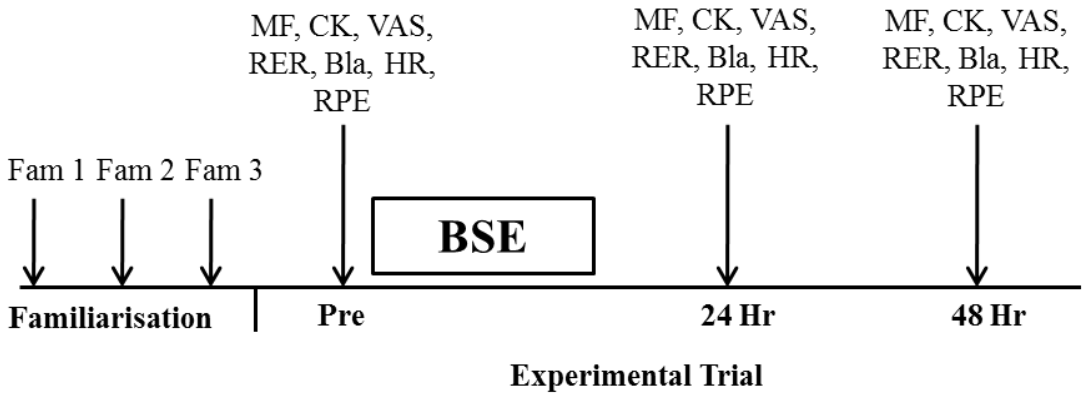

Figure 1. Schematic presentation of the experimental protocol. Indicating familiarisation sessions (Fam1, Fam 2 and Fam 3), measures of muscle function (MF) creatine kinase activity (CK), perceived soreness (VAS), respiratory exchange ratio (RER), blood lactate concentration (Bla), heart rate (HR) and ratings of perceived exertion (RPE) that were conducted pre bench stepping exercise and 24 and 48 hours post bench stepping exercise. The arrows indicate bouts of one legged cycling.

In the 48 hour period preceding the study, subjects maintained a diet comprising a balanced macronutrient 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, use of non-steroidal anti-inflammatory drugs 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.

Bench Stepping 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, Jones, & Edwards, 1983; Newham, Jones, Tolfree, & Edwards, 1986). 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. 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. 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 is a multi-joint movement pattern similar to cycling and that it isolates the desired muscle actions (concentric v eccentric) allowing for sufficient overloading of the force producing muscles 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 and concentric contractions of the quadriceps muscles of both the Ecc and the Con legs. Isometric force was measured at a knee angle of 75° (1.31 rad). Concentric torque was measured at an angular velocity of $30°s^{-1}$ ($0.52\ rad.s^{-1}$). A 60° (1.05 rad) range of motion was set whilst seated on the dynamometer, from maximal knee flexion using the dynamometers inbuilt goniometer. 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 experimental cycling session consisted of cycling with one leg for two 5 minute stages of fixed intensity. Prior to these two experimental stages, participants 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 at a self-selected cadence to allow the leg to become accustomed to the increase in blood flow to the working muscles. Participants then performed the two 5 min experimental stages and were encouraged to maintain their selected cadence at all times. These two workloads (WL1 and WL2) were 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). Heart rate (HR) was monitored continuously (Polar FS1, Polar Electro Oy, Finland), and

RPE was recorded using the Borg scale during the final 10s of WL1 and WL2 (Borg, 1970). Exactly the same protocol was followed during each experimental session.

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.

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 $\mu g \cdot kg^{-1} \cdot minute^{-}$ (Romijn et al., 1993).

Blood sampling

Approximately 30 μL of capillary blood was collected from a finger puncture 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 in the same manner 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 & Eston, 1992). 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, variables that were log transformed for analysis were CK and blood lactate. 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 using Mauchly's test. 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 ≤ to 0.05.

Results

Baseline comparisons

There were no significant differences between legs for any dependent variable of interest prior to the intervention (Table 1). Significant treatment × time and treatment × time × workload interactions reported below thus reflect effects of the eccentric exercise, and effects of the eccentric exercise as they interact with the increase in workload during the one-legged cycling exercise.

Skeletal muscle function

There were significant treatment × time interactions for measures of muscle function (*P*=0.006; *P*=0.039; concentric and isometric performance respectively, showing prolonged decrements (26% and 18% decrement in concentric and isometric forces respectively at 48 hour) in force generating capacities throughout the duration of the trial (Table 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=0.000). For both measures of perceived soreness (stepping up and down) an interaction of treatment × time (P=0.000) was observed with greater levels of soreness being evident in the Ecc condition and further increased at 48 hours (Table 1).

Insert Table 1

Creatine kinase

Capillary blood CK activity increased over time (P=0.000) with all post-exercise values elevated above baseline levels (Figure 2). The peak mean CK value recorded occurred 48 hours after the bench stepping exercise at 468±334 $IU.L^{-1}$ (range 80 – 1510 $IU.L^{-1}$).

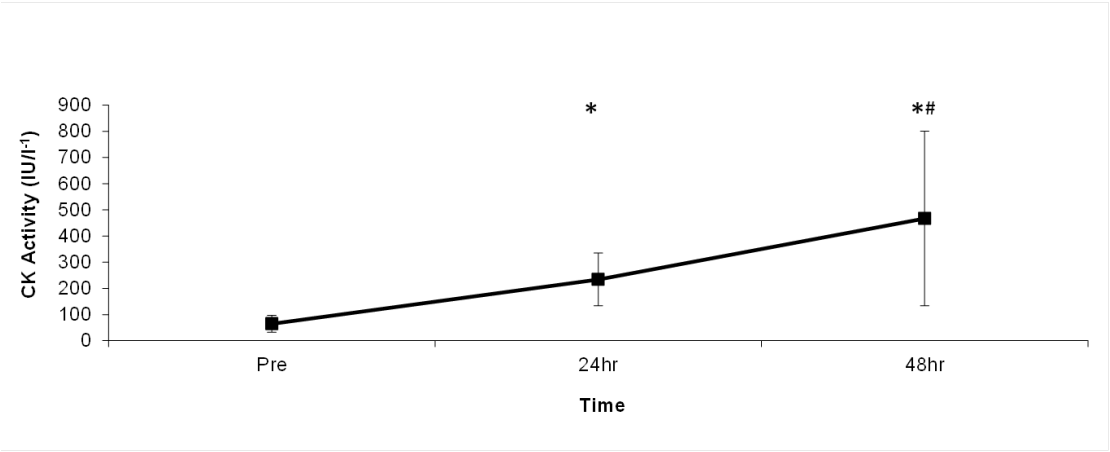

Figure 2. CK activity following bench stepping exercise (n=8). Data are mean(±**SD**). *Indicates significantly different from pre. # Indicates significantly different from 24 hour measure (P<0.05).

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 interaction (P=0.005), indicated that the effect of the treatment altered as workload increased. Accordingly, 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.

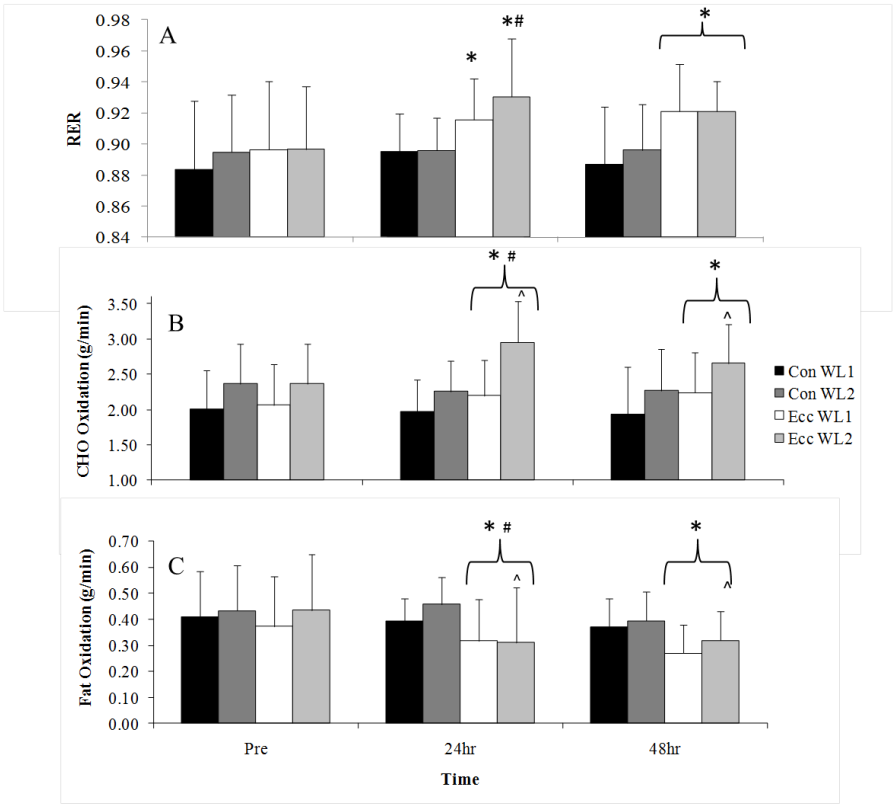

Figure 3. Respiratory exchange ratio (A), CHO oxidation (B) and fat oxidation (C) in Con and Ecc legs following bench stepping exercise (n=8). Data are mean(±SD). * Indicates significantly different from concentric trial ($p<0.05$). # Indicates significantly different from pre-measure ($P<0.05$). ^ Indicates significantly different from prior workload ($P<0.05$).

A main effect of effort (P=0.000) 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 for HR response during the one-legged cycling (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 was observed for RPE during the one-legged cycling (P=0.004), indicating that participants found it harder to cycle following Ecc than Con bench stepping exercise (Table 1).

Discussion

The main purpose of this study was to investigate whether EIMD could induce measurable changes in whole body substrate utilisation during subsequent concentric exercise. Completion of the bench stepping exercise resulted in significant decreases in muscular performance measures in the eccentrically exercised leg only (Table 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 EIMD that has been widely documented using similar protocols (Newham et al., 1983; Vissing, Overgaard, Nedergaard, Fredsted, & Schjerling, 2008). This demonstrates that the bench stepping exercise resulted in exercise induced damage in one leg (Ecc), but not in the other (Con), despite the matching of external work done.

Because circulating substrate availability would have been the same when cycling with either leg, the observed increase in the rate of CHO utilisation in Ecc suggests that damaged muscle has an increased reliance upon glycogen oxidation. It is possible that EIMD 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. Given the localised and focal nature of damage to type II muscle cells, this may be less of a contributor to the reduction in whole muscle

glucose uptake than has been proposed. 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, Amaral, & Caldwell, 1988). 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 EIMD.

Previously, it has been shown that intramyocellular triglyceride (IMCL) content is higher in damaged muscle compared with a work-matched concentrically exercised leg 24 hours following strenuous eccentric exercise (Hughes et al., 2010). It has been shown that IMCL will accumulate in situations where muscle glycogen cannot (Zderic, Davidson, Schenk, Byerley, & Coyle, 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 EIMD (Asp et al., 1998; Asp et al., 1995; Costill et al., 1990; Widrick et al., 1992). 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, Slotboom, Hoppeler, & Kreis, 1997; Gollnick, Piehl, Saubert, Armstrong, & Saltin, 1972; Hargreaves, McConell, & Proietto, 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. This may be explained by the larger metabolic cost for the concentric work due to tension being

created by the contractile units only. In contrast, during eccentric exercise, the 'Size Principle' does not apply (Nardone, Romano, & Schieppati, 1989) and the contractile proteins absorb some of the work, enabling less cross-bridges to perform the equivalent work compared to concentric contractions, thereby 'sparing' intramuscular stores of glycogen for subsequent work to be performed by intact muscle fibres.

EIMD 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 EMID. Recently it has been hypothesised that EIMD causes alterations in homeostasis through the requirement of energy to repair muscle tissue (Dolezal, Potteiger, Jacobsen, & Benedict, 2000). This has been confirmed in number of studies (Chesley, MacDougall, Tarnopolsky, Atkinson, & Smith, 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 & 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.

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 is based on a combination of muscular pain and the sensation of breathlessness (Jameson & 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, McDaniel, & Martin, 2010). This can be supported with the increased ratings of pain, as measure by visual analogue scale, in the eccentrically exercised leg 24 and 48 hours following the bench stepping exercise. This coupled with the increases in CK activity across the same time scale, indicate that the muscle damage imparted on the participants in the current study would be influenced to a greater extent by peripheral structural damage.

The main findings of the study 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 make more informed decisions regarding advice given to any population. Recreationally active individuals should be aware of the potential alterations to their performance and recovery capacity in the days following eccentric based exercise.

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.

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Table 1. Measures of knee extensor muscular strength and perceived muscle soreness (VAS – visual analogue scale), heart rate (Hr), blood lactate concentration (bla) and ratings of perceived exertion (RPE) during each workload (WL) in one-legged cycling following strenuous eccentric exercise (n=8). Data are mean(±**SD**). *Indicates significantly different from concentric leg. †Indicates significantly different from pre-measure (P<0.05). #Indicates significantly different from prior workload (P<0.05).

	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)*†	
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)*†	
	WL		**WL**		**WL**		**WL**		**WL**		**WL**	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Hr (bpm)	132(19)	141(16)	131(18)	141(16)	127(17)	138(15)	133(21)	141(20)	137(17)*	150(16)*#	134(17)*	146(15)*#
Bla (mmol·l^{-1})	3.7(1.6)	4.3(1.9)#	3.7(1.8)	4.1(1.7)#	3.5(2)	4.5(2.3)#	4.0(1.8)	4.4(2.7)#	3.6(1.2)	4.8(2)#	3.5(1.2)	4.3(1.4)#
RPE	13(3)	14(3)	12(3)	14(3)	13(3)	14(3)	13(3)	15(3)	14(3)*	16(3)*#	14(3)*	15(3)*#