1   **Title:** Neuromuscular responses to mild-muscle damaging eccentric exercise in a low glycogen state

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Neuromuscular responses to mild-muscle damaging eccentric exercise in a low glycogen state

Abstract

The aim of this study was to examine the effect of low muscle glycogen on the neuromuscular responses to maximal eccentric contractions. Fourteen healthy men (22±3 yr) performed single-leg cycling (20 min at ~75% maximal oxygen uptake (VO_{2max}); eight 90 s sprints at a 1:1 work-to-rest ratio (5% decrements from 90 to 55% VO_{2max}); and ~85% VO_{2max} until exhaustion) the evening before 100 eccentric knee extensions (1.57 rad·s^{-1}) with reduced (RED) and normal glycogen (NORM). Neuromuscular responses were measured during and up to 48 h after with maximal voluntary and involuntary (twitch, 20 Hz and 50 Hz) isometric contractions. During eccentric contractions, peak torque decreased (RED: -16.1±2.5%; NORM: -6.2±5.1%) and EMG frequency increased according to muscle length. EMG activity decreased for RED only. After eccentric contractions, maximal isometric force was reduced up to 24 h for NORM (-13.5±5.8%) and 48 h for RED (-7.4±10.9%). Twelve hours after eccentric contractions, twitch force and the 20:50 Hz ratio were decreased for RED but not for NORM. Immediate involuntary with prolonged voluntary force loss suggests that reduced glycogen is associated with increased susceptibility to mild muscle-damaging eccentric exercise with contributions of peripheral and central mechanisms to be different during recovery.

Introduction

Eccentric contractions are common for the knee extensors in daily activities, whether unaccustomed (such as downhill running) or habitual (such as stair descent). Vigorous eccentric contractions induce muscle damage, characterised by muscle soreness (Clarkson and Sayers, 1999), force loss (Behrens et al., 2012) and altered muscle glycogen metabolism (Asp et al., 1998; Widrick et al., 1992). Impairments in force-producing capacity have been attributed to central and peripheral neuromuscular origins (Edwards, 1988). Central mechanisms occur between the motor cortex and proximal to the neuromuscular junction and refer to the failure of central activation in muscle recruitment during contraction. Peripheral mechanisms reside below the neuromuscular junction and are associated with excitation-contraction (E-C) coupling failure due to: i) disturbed Ca^{2+} homeostasis (Warren et al., 2001; Verburg et al., 2006), and ii) sarcomeric disruption (Morgan et al., 2004). Furthermore, unique neuromuscular characteristics are exhibited for eccentric exercise, including reduced activation (Tesch et al., 1990) and early type II fibre recruitment (Nardone et al., 1989), with preserved recruitment order (Beltman et al., 2004). Following eccentric exercise, low-frequency fatigue (LFF) suggests disturbance in E-C coupling (Skurvydas et al., 2006) and intramuscular Ca^{2+} homeostasis (Verburg et al., 2006). LFF is characterised by force loss at low-, as opposed to high stimulation frequencies (Verburg et al., 2006).

Previously, decreased sarcoplasmic reticulum Ca^{2+} release, and thus reduced tetanic Ca^{2+} concentration, have been attributed to the emergence of LFF (Westerblad et al., 1993). Therefore, with force production associated with intramuscular Ca^{2+} release/uptake, muscle glycogen reduction is likely to present a metabolic stress, impairing sarcoplasmic reticulum function during subsequent exercise.

Neuromuscular origins contributing to fatigue and muscle damage have been studied widely. Some have reported little evidence of central mechanisms (Bigland-Ritchie et al., 1986; Kent-Braun, 1999), whereas others have demonstrated significant central involvement in response to knee extensor fatigue (Stewart et al., 2008). As maximal eccentric contractions produce forces exceeding isometric and concentric actions (Tesch et al., 1990), accompanying reduced neural drive indicates an inhibitory response, preventing further muscle-tendon unit damage (Clarkson and Sayers, 1999). Furthermore, metabolic consequences of eccentric exercise include disturbed Ca^{2+} homeostasis, preferential type II fibre damage (Asp et al., 1998; McHugh et al., 2000) and impaired glycogen recovery (Widrick et al., 1992). Maximal eccentric knee extensor exercise appears to compromise subsequent concentric exercise performance by increasing relative workload, and therefore utilising already reduced muscle glycogen stores (Asp et al., 1998). Mechanical and metabolic responses to knee extensor muscle damage are well characterised, however the neuromuscular responses during eccentric exercise with lowered muscle glycogen are unclear. Low muscle glycogen availability following prolonged exercise may be associated with susceptibility to, and recovery from muscle damage; and therefore relevant to those undertaking new training regimen or athletic cohorts performing heavy and/or repeat training bouts. Although neuromuscular responses to eccentric knee extensor exercise are well documented, it remains unresolved as whether an additive effect exists for exercise-induced mechanical and metabolic stresses on the i) recovery of neuromuscular function, and ii) the contributory mechanisms. This study protocol aimed to examine the effect of reduced muscle glycogen availability on the neuromuscular responses to maximal eccentric contractions.

Methods

Participants

Fourteen healthy males (age, 22±3 years; height, 179±6 cm; weight, 76±15 kg; body fat, 9±3%) volunteered and provided written informed consent for participation. Participants were recreationally active, free from musculoskeletal injury and had not been involved in, or were presently engaged in resistance training. Sample size estimation was based on data for maximal voluntary contraction (MVC) force loss (-19.8%, α level = 0.05,

1   power = 0.80 (1 − β) (Faul et al., 2007). The research protocol was approved by the University of Chichester

2   Research Ethics Committee.

3

4   *Study protocol*

5   Participants visited the laboratory on seven occasions within 10 days. Visits one and two were familiarisation

6   sessions to accustom participants with single-leg cycling, electrical stimulation procedures, and to establish

7   EMG sensor placement. Visit three was a pre-eccentric, evening session involving an exercise-diet intervention

8   to manipulate glycogen state. With dietary control, this involved single-leg cycling to reduce muscle glycogen in

9   the right leg; the resting left leg served as control. The protocol was validated in a similar cohort by directly

10   measuring muscle glycogen (muscle glycogen reduced by ~44.7%) (Pilegaard et al., 2002). Visit four followed

11   early the next morning, with participants performing 100 maximal, eccentric knee extensions with the reduced

12   glycogen availability (RED) right leg, and then the normal glycogen availability (NORM) left leg. Visits five to

13   seven involved assessment of neuromuscular function and muscle soreness at 12 h intervals, up to 48 h.

14   Neuromuscular assessment also occurred immediately after single-leg cycling, for the RED leg, and then the

15   NORM leg. Aside from visits three and four, where diet was manipulated to limit glycogen resynthesis,

16   volunteers were instructed to arrive 3 h after their final meal and caffeine intake. Water was provided *ad libitum*.

17   Throughout the experimental period, participants were requested to avoid strenuous physical activity.

18

19   *Familiarisation*

20   Familiarisation one commenced with a maximal oxygen uptake ($\dot{V}O_{2max}$) test on a two-leg cycle ergometer

21   (Excalibur Sport 925900, Lode, Groningen, the Netherlands) to determine single-leg cycling workload. After a

22   10 min warm up, participants cycled at 80 W for 60 s (~80 rpm), thereafter workload increased by 30 W every

23   60 s until volitional exhaustion. A 15 min rest was then followed by submaximal, single-leg cycling to adjust the

24   cycle ergometer settings to the individual, and to calculate participant-workload based upon two-leg $\dot{V}O_{2max}$

25   values. The right leg cycled (70 to 80 rpm) unloaded for 10 min, then 3 min load increments were introduced

26   until individual workload corresponding to 75% two-leg $\dot{V}O_{2max}$ was maintained. This ~30 min procedure was

27   followed by a 15 min rest, and then the electrical stimulation protocol to determine maximal (right leg, 657±98

28   mA; left leg, 670±110 mA) and submaximal (5% MVC) currents (right leg, 111±24 mA; left leg, 114±17 mA) to

29   be used in subsequent assessments. Forty-eight hours later, familiarisation two involved further electrical

30   stimulation and single-leg cycling practice, and then isokinetic dynamometer and EMG configuration.

1

2 *Glycogen reduction exercise*

3 Participants arrived at the laboratory between 19:00 and 20:00 h in a 3 h fasted state, to perform exhaustive,

4 single-leg cycling. The left crank was removed from a cycle ergometer (Monark Ergomedic 824E, Varberg,

5 Sweden) and replaced with a platform to support the resting left leg. The right foot was strapped to the pedal of

6 the right crank, and the left leg was secured in a relaxed position on the support stand. Maximal single-leg

7 cycling workload was calculated as 74% of maximal two-leg workload (Pernow and Saltin, 1971) achieved in

8 the familiarisation cycling trial. Subsequent workload calculations for single-leg cycling were derivatives of

9 single-leg maximal workload, and referred to henceforth. Respiratory responses (Table 1) were measured using a

10 portable metabolic system (Cosmed K4b^2, Rome, Italy). Single-leg cycling involved: 20 min at 75% $\dot{V}O_{2max}$

11 power (2.3±0.3 kg; 178±27 W); eight 90 s sprints at a 1:1 work-to-rest ratio (5% decrements from 90 to 55%

12 $\dot{V}O_{2max}$); and exhaustive cycling at 85% $\dot{V}O_{2max}$ for the right limb (Pilegaard et al., 2002). Cadence was

13 maintained between 70 and 80 rpm; exhaustion was confirmed with an inability to sustain 50 rpm. To limit

14 localised glycogen resynthesis in the RED leg, 30 min of arm cranking exercise (Monark 864, Varberg, Sweden)

15 followed single-leg cycling (Camera et al., 2012; Pilegaard et al., 2002). Finally, a low carbohydrate diet was

16 provided (~3932 kJ: protein ~960 kJ, carbohydrate ~70 kJ, fat 2907 kJ) consisting of three eggs and six bacon

17 slices, and for breakfast the next morning, two eggs (Steensberg et al., 2002).

18

19 <<< INSERT TABLE 1 HERE >>>

20

21 *Eccentric exercise*

22 The next morning (~06:30 h), maximal, eccentric knee extensions for the RED leg, and then the NORM leg were

23 completed. Contractions were performed in a non-randomised order to limit the time-course for glycogen

24 resynthesis in the RED leg, as resynthesis rate is elevated after exhaustive exercise (Casey et al., 1995). Ten sets

25 of ten eccentric contractions were performed (1.57 rad·s^{-1}), separated by 60 s rests (Byrne et al., 2001) on an

26 isokinetic dynamometer (Humac Norm, Cybex International Inc., NY, USA). Seated and secured upright (with

27 the hip joint at 1.57 rad) in the dynamometer chair; the lateral femoral epicondyle of the contracting leg was

28 aligned with the rotational axis of the gravity corrected dynamometer and the lever arm was fastened to the ankle

29 joint. Instruction was given to resist the lever arm moving through a 0.09 to 1.74 rad range of motion (ROM)

30 (full knee extension = 0 rad). Standardised verbal encouragement, and instantaneous torque-time curve feedback

1   from the dynamometer monitor, were provided to ensure maximal effort throughout entire ROM. Peak torque

2   and negative work done per eccentric set were normalised to mean of eccentric set one.

3

4   *Surface electromyography recording and processing*

5   *Vastus lateralis*, *vastus medialis* and *rectus femoris* EMG were recorded via bipolar sensors, from a Delsys

6   Bagnoli-8 system (Delsys Inc., Boston, USA) during eccentric exercise. To minimise skin-to-electrode

7   impedance, the skin was shaved, cleansed and abraded. Sensors were fixed on the mid-aspect of the muscle belly

8   according to recommendations (Hermens et al., 2000). The reference electrode was placed approximately 4 cm

9   proximal to the patella apex of the assessed leg. Signals were sampled at 1000 Hz by amplifiers embedded in the

10   EMG sensors (10 x 1 mm sensor contacts, 10 mm inter-electrode distance; bandwidth = 20-240 Hz; common

11   mode rejection ratio = 92 dB; input impedance = >1015 Ω). Raw EMG data were then processed with a 2nd order

12   Butterworth filter (bandwidth of 10-350 Hz), and the root mean square (RMS) accounting for time-phase shifts

13   (EMGworks® Data Acquisition and Analysis software, Delsys Inc., Boston, USA). Filtered data were also

14   treated with a fast Fourier transformation to quantify median frequency (MDF). To ensure isovelocity and

15   examine EMG activity at different stages of the contraction mid-range, peak torque, RMS and MDF were

16   calculated for three, 0.26 rad epochs. The first and final 0.48 rad ranges were excluded from analysis due to: i)

17   some participants being unable to maintain a constant contraction velocity at range of motion limits, and ii) time

18   for the lever arm to reach constant angular velocity (Fig. 1). Epochs corresponded to estimates of short (first;

19   0.48 to 0.74 rad), medium (second; 0.74 to 1.0 rad) and long muscle lengths (third; 1.0 to 1.26).

20

21   <<< INSERT FIG. 1. HERE >>>

22

23   *Electrical stimulation and muscle soreness*

24   Knee extensor neuromuscular function was measured on a custom-built chair, with a DS7A electrical stimulator

25   and NeuroLog pulse generator (Digitimer Limited, Welwyn Garden City, UK). Participants were seated and

26   secured, (hip and knee at 1.57 rad), with the ankle joint attached via a steel chain leading to a mechanically

27   calibrated load-cell (RS 250 kg, Tedea Huntleigh, Cardiff, UK) at the chair base. Electrical stimulation was

28   received through two saline treated electrodes (9 x 18 cm) positioned over the proximal and distal part of the

29   thigh. Muscle soreness was reported at rest using a visual analogue scale (0 = not at all sore; 10 = extremely

30   sore), with the muscle-belly palpated until enough pressure was exerted to blanch the fingernail. Warming up

1   involved three 5 s isometric, submaximal knee extensions and standardised stretching. Maximal twitch was

2   ascribed, progressively applying 50 mA pulses until the twitch force reached a plateau. Unlike nerve stimulation,

3   muscle stimulation with large electrodes may involve antagonist co-activation and incomplete agonist activation,

4   yet it presents less discomfort and can be considered a valid alternative to nerve stimulation (Place et al., 2010).

5   Submaximal twitch was then calculated (equivalent to 5% isometric MVC force), and measured by delivering a

6   single, 1 ms stimuli (100 mA, 400 V) to the relaxed knee extensors. Then, participants produced three separate, 3

7   to 5 s isometric MVC (2 min rests), with verbal encouragement and instantaneous force-time feedback from a

8   computer monitor. After a 2 min rest, 20 and 50 Hz stimulations (0.5 s duration) were applied using the

9   submaximal current. Ratio between 20 and 50 Hz forces was calculated; a decrease of which indicates LFF.

10

11   *Statistical analysis*

12   Two-way repeated measure ANOVA was used to examine condition-set interaction (RED and NORM) for

13   neuromuscular responses during and after eccentric exercise. A post-hoc Bonferroni correction was used to

14   control for multiple comparisons. Eccentric exercise responses (peak torque, negative work done, RMS and

15   MDF) were compared between contractions one and one-hundred for conditions (RED and NORM), separately

16   for each three epochs. Effect sizes (Cohen's *d*) were calculated to detect meaningful difference; values are

17   interpreted as 0.2 for small, 0.5 for moderate, and 0.8 for large differences. Data are expressed as means±SD,

18   with statistical significance set as $P < 0.05$.

19

20   **Results**

21   *Peak torque and negative work*

22   Baseline peak torque was 195.8±32.9 N·m for RED, and 178.2±26.8 N·m for NORM (P=0.01), respectively.

23   Peak torque showed a condition-set interaction ($F_{(1,13)}$=11.9, P=0.0001, *d*=0.57), decreasing by -16.1±2.5%

24   (P=0.0001) for RED, and -6.2±5.1% (P=0.004) for NORM during eccentric contractions (Fig. 2). Peak torque

25   decreased at short muscle length for both legs (RED, P=0.02, -19.3±2.8 %, *d*=0.6; NORM, P=0.01, -18.4±2.2%,

26   *d*=0.6), and at long muscle length (P=0.02, -21.7±1.9%, *d*=0.63) for RED. Negative work decreased during

27   eccentric contractions ($F_{(1,13)}$=4.9, P=0.05, *d*=0.27) by -11.2±1.4% (P=0.002, *d*=0.4) for RED, but not for

28   NORM (P=0.6, 3.1±0.6%, *d*=-0.09) by set ten.

29

30   <<< INSERT FIG. 2. HERE >>>

1

2 *Electromyography responses*

3 *Rectus femoris* RMS was unchanged during eccentric contractions for RED at short (P=0.1, -2.6±1.7%, Fig. 3)

4 and medium muscle lengths (P=0.07, -9.6±2.8%), but increased at long muscle length (P=0.03, 16.2±4.1%,

5 *d*=0.22). *Rectus femoris* RMS was unchanged during eccentric contractions for NORM at all muscle lengths.

6 *Vastus lateralis* ($F_{(1,13)}$=6.0, P=0.08, *d*=0.35) and *vastus medialis* ($F_{(1,13)}$=1.79, P=0.07, *d*=0.13) RMS were

7 unchanged for both conditions.

8

9 <<< INSERT FIG. 3. HERE >>>

10

11 *Rectus femoris* MDF increased at short muscle length for RED (P=0.01, 13.2±4.0%, *d*=0.56) and at long muscle

12 length for both conditions (RED: P=0.02, 17.9±3.6%, *d*=0.85; NORM: P=0.02, 15.5±3.2%, *d*=0.81). *Vastus*

13 *medialis* MDF differed between conditions ($F_{(1,13)}$=1.98, P=0.04, *d*=0.18), unchanged for RED, but decreased for

14 NORM (P=0.006, -16.3±4.5%, *d*=0.83) during eccentric exercise. *Vastus lateralis* MDF decreased ($F_{(1,13)}$=2.47,

15 P=0.01, *d*=0.25) for both conditions during eccentric exercise (RED: P=0.01, -20.7±6.2%, *d*=0.8; NORM:

16 P=0.003, -15.8±5.2%, *d*=0.55).

17

18 *Neuromuscular recovery*

19 Single-leg cycling decreased MVC ($F_{(1,13)}$=13.9, P=0.0001, *d*=0.50) for RED (P=0.0001, -23.2±9.2%, *d*=1.20)

20 and NORM (-5.4±5.3%, P=0.002, *d*=0.30) from pre-condition. Eccentric contractions decreased MVC in both

21 conditions at 0 h (RED: P=0.0001, -23.9±12.0%, *d*=1.42; NORM: P=0.0001, -19.4±5.6%, *d*=1.15), 12 h (RED:

22 P=0.0001, -10.6±12.1%, *d*=0.58; NORM: P=0.01, -9.7±6.6%, *d*=0.57) and 24 h (RED: P=0.0001, -15.8±10.3%,

23 *d*=0.84; NORM: P=0.0001, -13.5±5.8%, *d*=0.78). At 48 h MVC was decreased for RED only (P=0.003, -

24 7.4±10.9%, *d*=0.36; Fig. 4a). After single-leg cycling, twitch force decreased ($F_{(1,13)}$=7.7, P=0.001, *d*=0.39) for

25 RED (P=0.001, -46.7±14.5%, *d*=1.47) and increased for NORM (P=0.05, 20.9±14.4%, *d*=-0.64). Eccentric

26 contractions decreased twitch for RED (P=0.0001, -41.3±9.1%, *d*=1.95), but not NORM (P=0.2, -13.7±8.7%,

27 *d*=0.44; Fig. 4b). At 12 h, twitch force had recovered for both conditions.

28

29 <<< INSERT FIG. 4. HERE >>>

30

1 Single-leg cycling decreased 20:50 Hz force ratio ($F_{(1,13)}$=14.0, P=0.0001, d=0.52) for RED only (P=0.0001,

2 d=1.30). Eccentric contractions decreased 20:50 Hz ratio for both conditions (RED: P=0.003, 0.75±0.09 to

3 0.64±0.11, d=0.93; NORM: P=0.01, 0.76±0.12 to 0.66±0.09, d=0.79). The 20:50 Hz ratio was decreased at 12 h

4 for RED only (P=0.03, 0.70±0.09, d=0.38; Fig. 4c). At 24 h the 20:50 Hz ratio had recovered in both conditions.

5 Muscle soreness increased ($F_{(1,13)}$=0.64, P=0.03, d=0.17) after single-leg cycling for both conditions (RED:

6 P=0.006, 0.7±0.3, d=0.96; NORM: P=0.01, 0.5±0.4, d=0.72), and immediately after eccentric contractions

7 (RED: P=0.001, 2.2±0.5, d=0.85; NORM: P=0.03, 1.5±0.6, d=0.68). Soreness remained up to 48 h in both

8 conditions (P>0.05, RED: 2.0±1.1, 2.1±0.9, 1.8±1.0; NORM: 1.8±1.0, 2.2±1.4, 2.0±0.8; Fig. 4d).

9

10 **Discussion**

11 This study examined the effect of muscle glycogen reduction on the neuromuscular responses to, and recovery

12 from mild exercise-induced muscle damage. Performance decline during eccentric contractions with RED was

13 accompanied by decreased *rectus femoris* and *vastus lateralis* EMG activity, and increased *rectus femoris* EMG

14 frequency. In recovery, voluntary force loss persisted for longer (up to 48 h), and immediate involuntary force

15 loss was greater for RED. Twitch force was recovered after 12 h, but 20:50 Hz ratio remained decreased for

16 RED. Persistent voluntary force loss, with short-term involuntary force loss following eccentric contractions

17 suggests peripheral and central mechanisms in early recovery, and then central-mediated mechanisms during

18 prolonged recovery with lowered glycogen.

19 Hody et al., (2013) found negative work during maximal eccentric knee extensions to be associated

20 with the magnitude of several indirect muscle damage markers. Therefore, the preservation of negative work by

21 the NORM leg during eccentric contractions is expected to induce greater neuromuscular damage. Conversely,

22 the greater immediate peripheral fatigue shown for RED may be partially explained by single-leg cycling

23 imposing metabolic stress, including disrupted Ca^{2+} homeostasis (Kent-Braun, 1999). That negative work done

24 did not account for induced damage can also be attributed to the high variability of symptoms reported by Hody

25 and colleagues (2013). These authors measured plasma creatine kinase activity, muscle soreness and stiffness,

26 whereas we assessed voluntary force, involuntary force and muscle soreness. We used single-leg cycling

27 previously shown to reduce muscle glycogen to 337±33 mmol·kg^{-1}·dry weight in the exercising leg (compared to

28 609±47 mmol·kg^{-1}·dry weight in the resting, control) (Pilegaard et al., 2002). Altered metabolic acidosis

29 following single-leg cycling, particularly lowered pH, would be likely to disturb muscle membrane excitability

30 and E-C coupling, subsequently incurring greater peripheral fatigue. Stewart et al., (2008) found cycling to

fatigue on three consecutive days decreased 10 Hz force after the first (-40%), and third bouts (-34%). For the

same time-points, MVC force was decreased by -14% and -11%, and 100 Hz force unchanged and decreased by

-16%. After eccentric contractions we found peripheral fatigue transient for RED, which did not account for

persistent neuromuscular impairment. Involuntary force losses were recovered 12 h after eccentric exercise, yet

more severe muscle damage would cause greater neuromuscular impairment with which to study glycogen-

mediated differences with greater sensitivity.

We found little change in muscle performance during eccentric contractions under normal conditions;

this concurs with Tesch et al., (1990) who showed torque loss between -34 and -47% for maximal, concentric

contractions, but minimal change for maximal, eccentric contractions. Therefore, the greater forces produced

during eccentric contractions are better preserved. Whereas muscle activity increased, and frequency decreased

with concentric actions, both were unchanged with eccentric (Tesch et al., 1990). Similarly, RMS was

unchanged for NORM, which was supported by maintained muscular performance. In the RED condition,

decreased RMS indicates reduced motor neurone firing, which may reflect an inhibitory response to prevent

further neuromuscular damage. In addition, range of motion data allowed analysis at constant angular velocity.

Decreased peak torque was observed from repetition one to one-hundred at short (-19.3%) and long muscle

lengths (-21.7%) for RED, but only at short length for NORM (-18.4%). Greater force loss at short lengths may

indicate a right-ward shift in muscle length-tension characteristics (Byrne et al., 2001) for NORM, but similar

loss at long length for RED, suggests single-leg cycling also altered contractile structures. Furthermore, peak

torque decreased at short and long muscle lengths for RED indicating advancement, or early onset of contractile

damage. As motor unit activation is incomplete for eccentric contractions (McHugh et al., 2000), NORM would

be expected to have a greater motor unit reserve, than RED, such that it could activate 'fresh' units during the

repeated contractions, thus postponing fatigue. Increased MDF during eccentric exercise indicates fatigue, and

may reflect additional recruitment of type II fibres with greater conduction velocity (Kamen and Caldwell,

1996), more so if single-leg cycling depleted type I fibres. We speculate that the higher *rectus femoris* MDF in

RED, than in NORM, during eccentric exercise would indicate higher conduction velocity and greater type II

fibre recruitment, as single-leg cycling preferentially utilised type I muscle fibres and their glycogen stores.

However, because eccentric contractions with RED resulted in unchanged *vastus medialis* MDF, and decreased

vastus lateralis MDF, we cannot find a robust association between low glycogen availability and muscle fibre

recruitment during eccentric exercise.

1   Following eccentric contractions, voluntary force production was decreased up to 24 h for NORM and

2   48 h for RED. Single-leg cycling did not incur lasting neuromuscular function, yet disturbed intramuscular

3   *milieu* may have activated group III and IV afferents in RED, more than NORM. Heightened perceptual

4   sensitivity could explain the larger effect sizes of soreness shown for RED after single-leg cycling and eccentric

5   contractions. Furthermore, afferent feedback is known to facilitate central fatigue (Amann et al., 2011), which is

6   consistent with our findings of decreased voluntary force (up to 48 h) and limited LFF (up to 24 h) shown for

7   RED. Low glycogen availability leads to a decreased ATP resynthesis rate, and therefore reduced intracellular

8   ATP. This compromises energy demand to satisfy E-C coupling, and results in an inability to convert neural

9   drive into force output (Green, 1991). In this study LFF remained up to 12 h post-eccentric in both conditions,

10   suggesting glycogen availability had little effect on E-C coupling disruption. However, the active limb

11   experienced decreased 20 (-47.9%) and 50 Hz forces (-32.8%) after single-leg cycling.

12   A limitation to the current study is the lack of biopsy/blood sampling, making it difficult to: i) confirm

13   muscle glycogen reduction, and ii) discern whether single-leg cycling resulted in structural and metabolic

14   changes in the RED leg. We are confident the pre-validated, single-leg cycling protocol reduced glycogen in the

15   RED leg, however biopsies would have provided direct assessment of glycogen reduction, and recovery.

16   Furthermore, it is likely that the neuromuscular differences between conditions were not solely due to muscle

17   glycogen concentration, but other structural and metabolic alterations caused by single-leg cycling. In terms of

18   measurement tools, EMG, muscle stimulation and indirect calorimetry are easily administered, non-invasive

19   methods to quantify neuromuscular behaviour.

20

21   **Conclusions**

22   During eccentric exercise, reduced muscle glycogen resulted in a failure to maintain muscular performance, and

23   a decreased muscle activity. In recovery, eccentric exercise with reduced glycogen caused greater, early evoked

24   force loss, delayed voluntary force recovery, independent of muscle soreness. Immediate involuntary force loss,

25   with prolonged voluntary force loss, indicates reduced glycogen is associated with both central and peripheral

26   mechanisms in early recovery and then central mechanisms in the later recovery. These results suggest that

27   reduced muscle glycogen impairs muscular work capacity and alters neuromuscular fatigue profiles following

28   mild exercise-induced muscle damage of the knee extensors. These findings have application to individuals

29   performing repeat training bouts and mechanistically, understanding the impact of performing damaging exercise

30   in a pre-fatigued state.

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1 **Tables**

2 **Table 1** Mean physiological responses to single-leg muscle glycogen reduction cycling.

	75% VO$_{2max}$				90%	85%	80%	75%	70%	65%	60%	55%	85% VO$_{2max}$
	5 min	10 min	15 min	20 min	90 s sprints (1:1 work-to-rest ratio)								Exhaustive
Workload (W)	124±30	126±29	126±32	138±29	162 ±33	152±34	147±34	140±36	130±37	125±34	121±29	121±25	134±35
Cadence (rpm)	71.9±5.2	70.4±3.0	69.7±3.7	68.6±7.4	74.8±6.2	74.8±5.1	76.6±4.7	76.6±5.8	77.8±7.4	78.9±6.0	80.8±6.6	81.0±7.6	66.1±7.7
V$_E$ (L·min^{-1})	76.2±14.3	87.7±21.6	93.2±18.7	93.7±21.8	92.6±16.6	98.5±19.8	92.3±20.7	87.4±19.5	86.8±20.3	80.1±19.2	72.9±20.7	72.4±20.1	102.2±23.0
VO$_2$ (L·min^{-1})	2.28±0.55	2.30±0.54	2.46±0.63	2.49±0.53	2.59±0.44	2.6±0.47	2.55±0.4	2.5±0.5	2.46±0.53	2.44±0.43	2.33±0.56	2.19±0.48	2.45±0.69
VCO$_2$ (L·min^{-1})	2.39±0.65	2.30±0.59	2.35±0.53	2.44±0.54	2.57±0.44	2.46±0.43	2.33±0.32	2.21±0.38	2.20±0.46	2.09±0.26	1.95±0.47	1.89±0.41	2.33±0.74
VO$_2$ (mL·kg·min^{-1})	29.9±8.9	30.0±8.4	32.0±9.1	32.4±7.7	33.8±7.2	34.0±7.5	33.3±6.6	32.5±7.2	32.2±7.7	32.1±7.3	30.3±7.5	28.7±6.8	31.6±9.0
RER	1.04±0.13	1.00±0.14	0.95±0.15	0.98±0.12	1.00±0.11	0.95±0.12	0.92±0.11	0.89±0.1	0.90±0.1	0.87±0.09	0.84±0.1	0.87±0.11	0.93±0.2

| HR (b·min^{-1}) | 170±14 | 165±17 | 159±21 | 153±20 | 171±15 | 172±14 | 170±16 | 170±12 | 166±12 | 162±12 | 160±13 | 157±12 | 177±8 |

1   Values are mean±SD; V_E, minute ventilation; VO_2, oxygen uptake; VCO_2, carbon dioxide production; RER, respiratory exchange ratio; HR, heart rate.
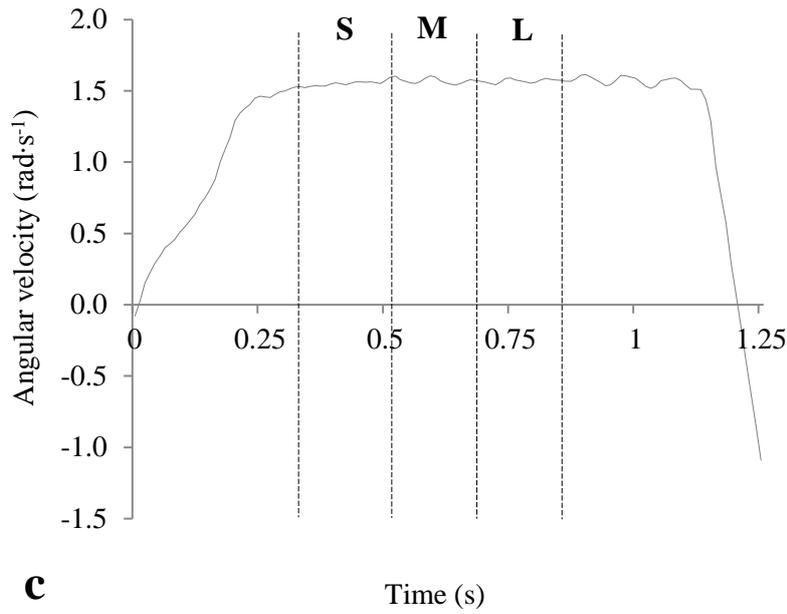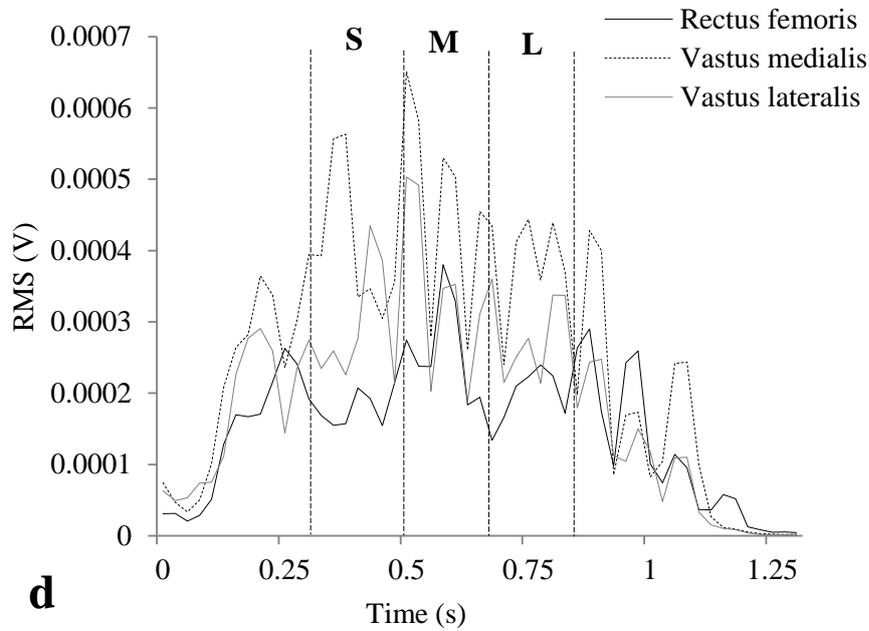
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2   **a**

b
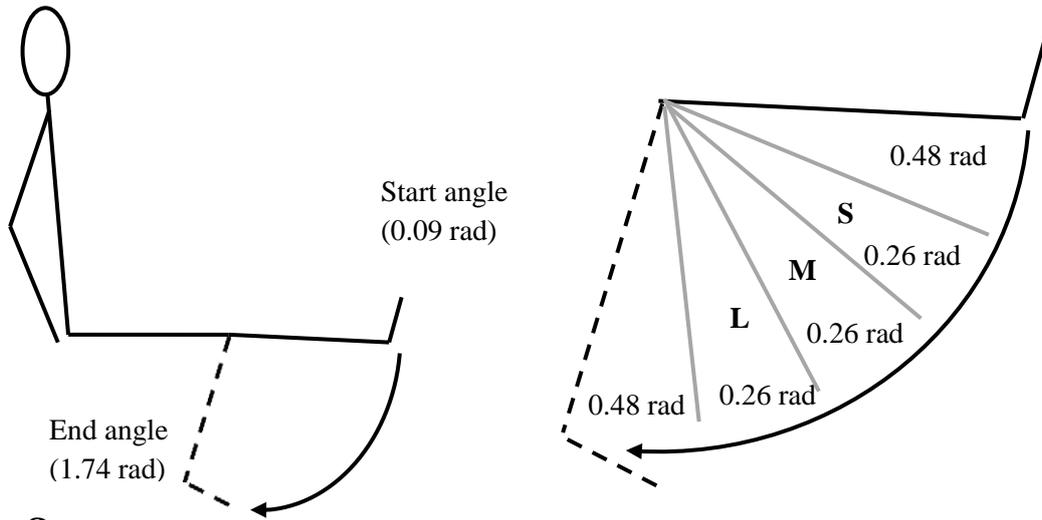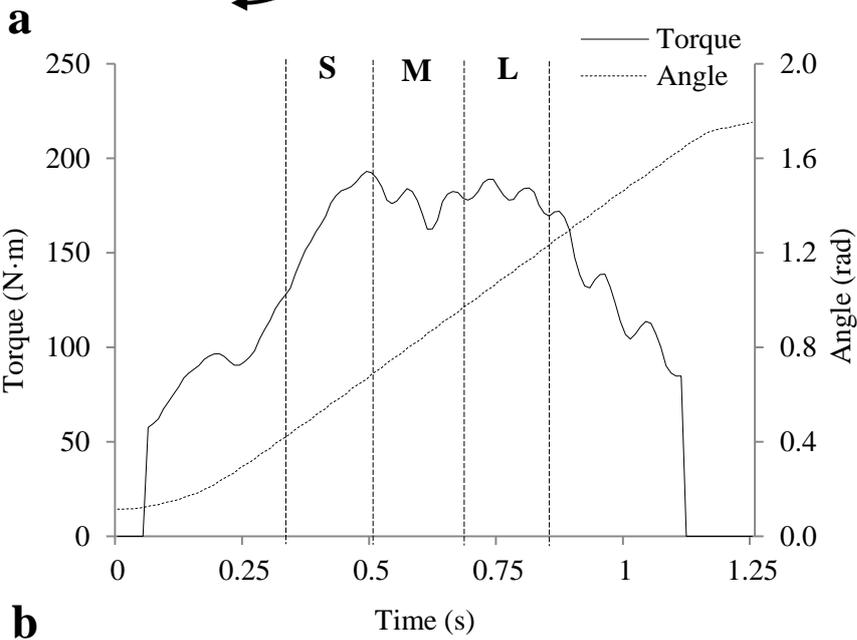
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Fig. 1. A schematic of the knee angles during maximal, eccentric knee extensions performed on an isokinetic dynamometer at 1.57 rad·s⁻¹ (a). To ensure isovelocity and examine the influence of muscle length, peak torque (b), angular velocity (c), EMG root mean square (RMS) (d) and median frequency (MDF) recorded during eccentric contractions were averaged across three, 0.26 rad epochs. With the first and final 0.48 rad excluded, epochs corresponded to short (**S**) (first; 0.48 to 0.74 rad), medium (**M**) (second; 0.74 to 1.0 rad) and long (**L**) muscle lengths (third; 1.0 to 1.26).
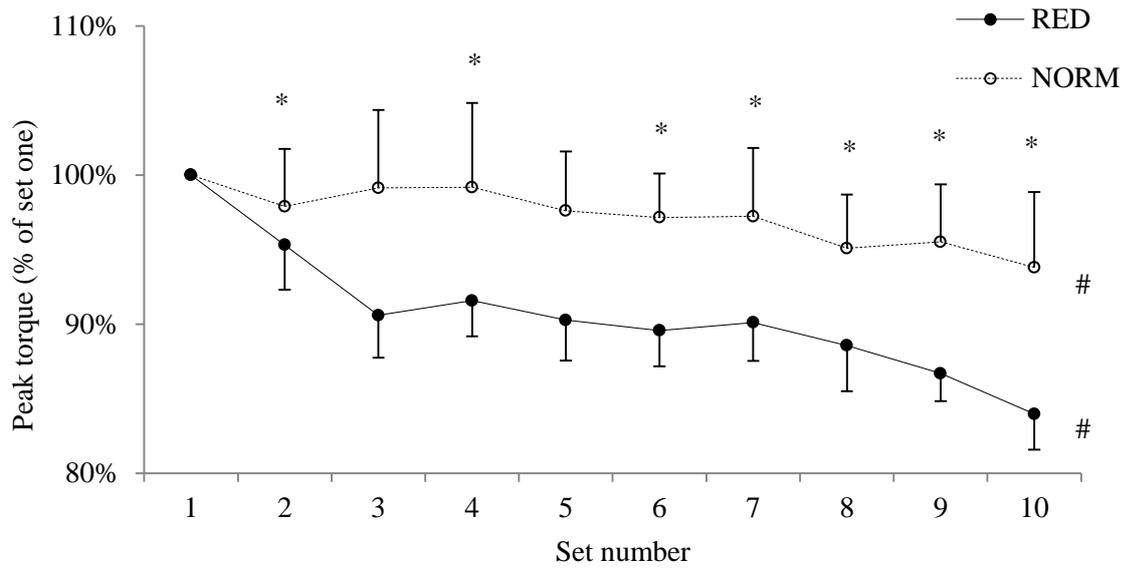
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2 **Fig. 2.** Peak torque (normalised to set one) during maximal eccentric knee extensions with reduced (RED) and

3 normal (NORM) glycogen availability legs. Data presented are mean±SD. * Significant difference between

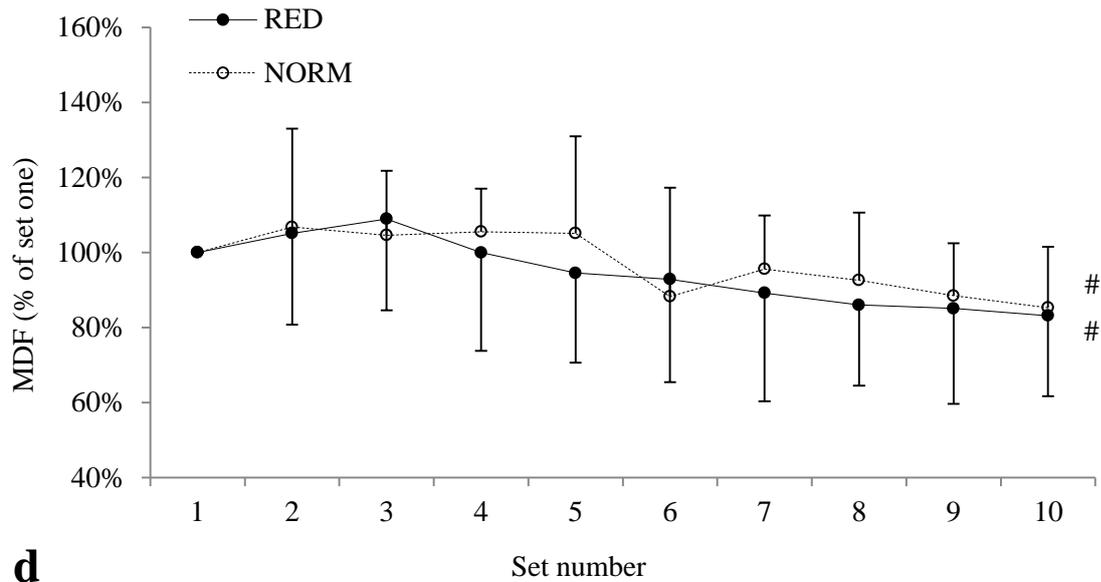4 conditions, # significant difference between sets one and ten, $P < 0.05$.

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a

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b

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c

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d
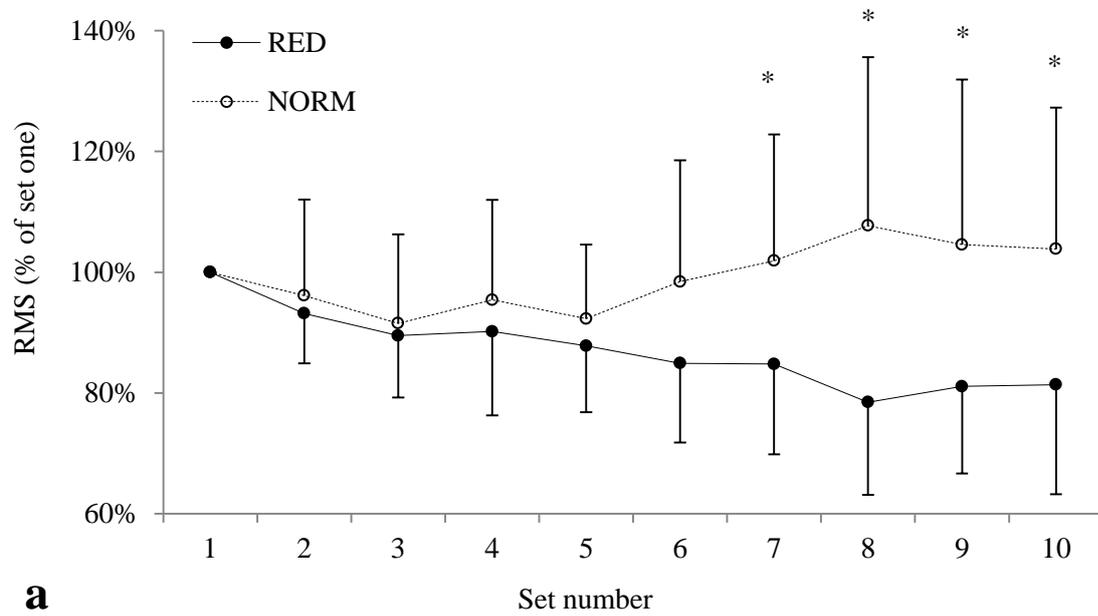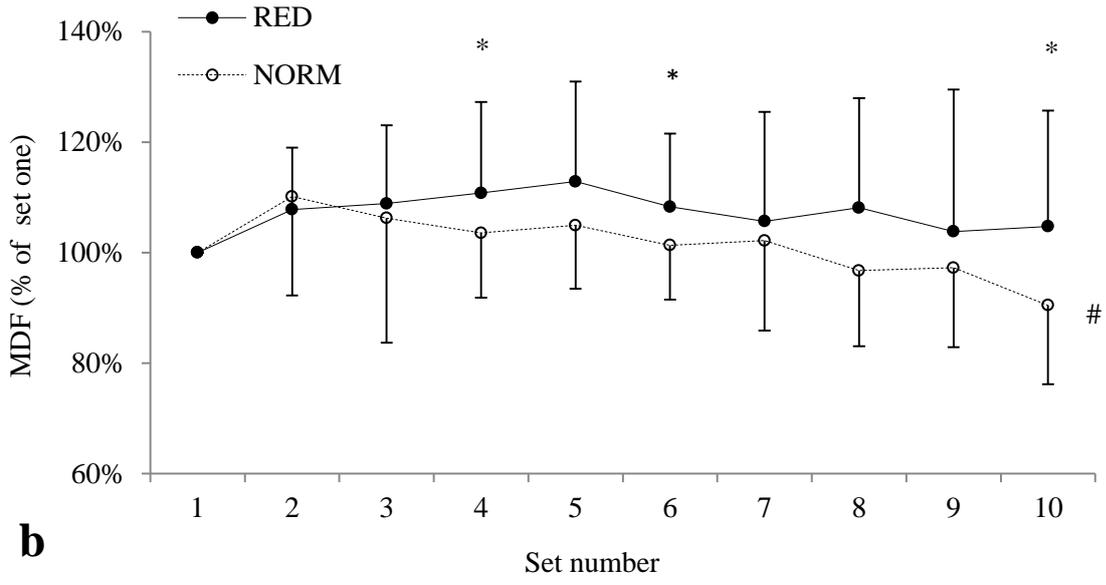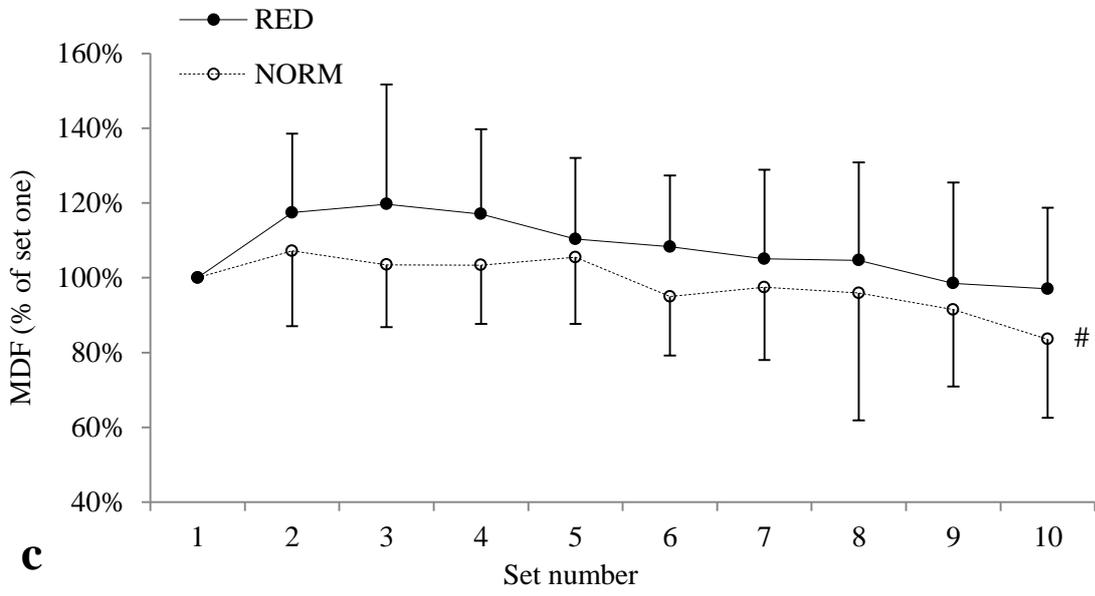
Fig. 3. *Rectus femoris* root mean square (RMS) (a) and median frequency (MDF) (b), and *vastus medialis* MDF (c) and *vastus lateralis* MDF (d) during maximal eccentric knee extensions with reduced (RED) and normal (NORM) glycogen availability legs. Data presented are mean±SD. * Significant difference between conditions, # significant difference between sets one and ten, P < 0.05. MDF data refer to total epoch mean, not individual epoch mean.

a
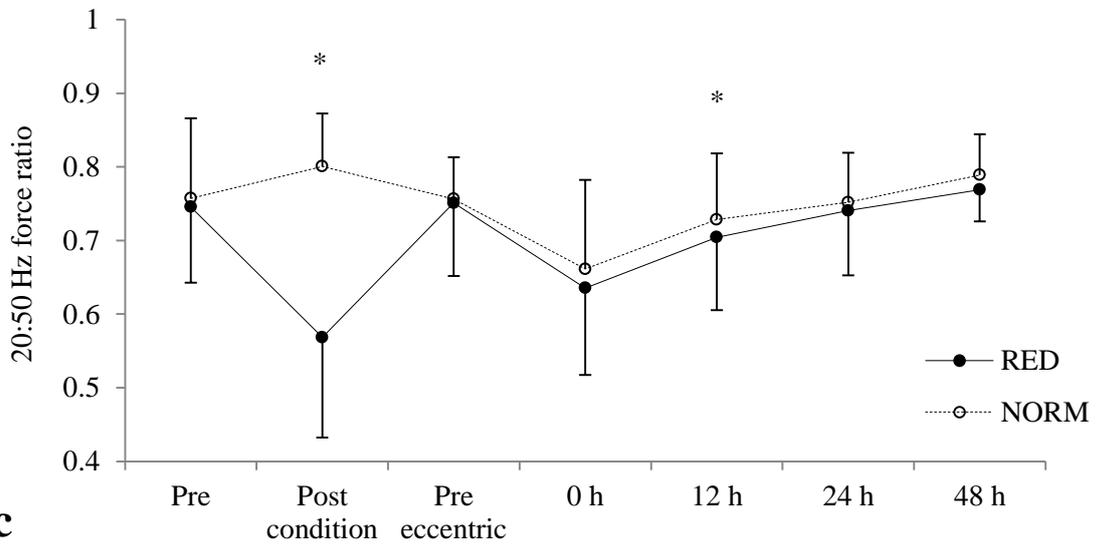
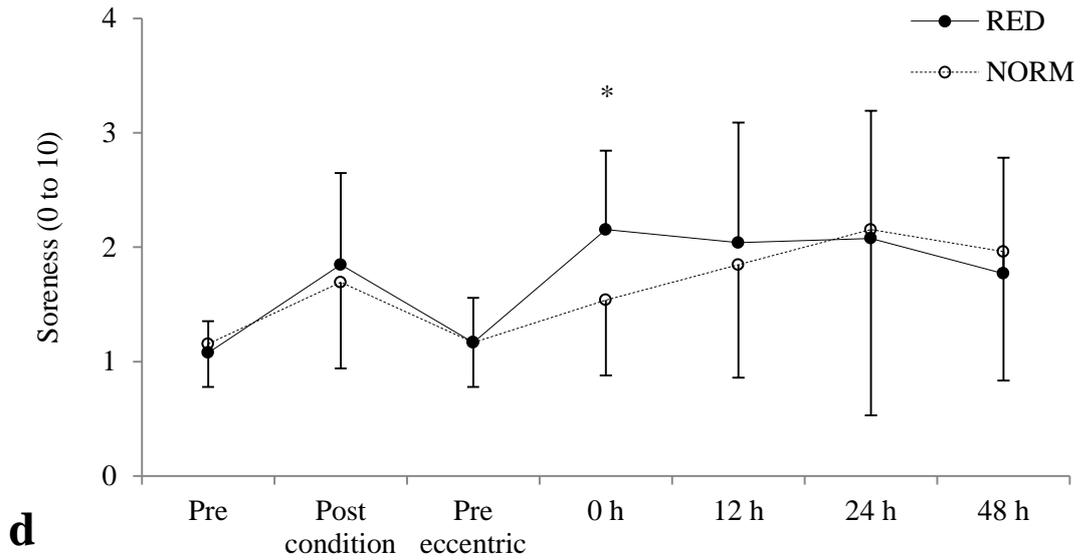**b**

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c

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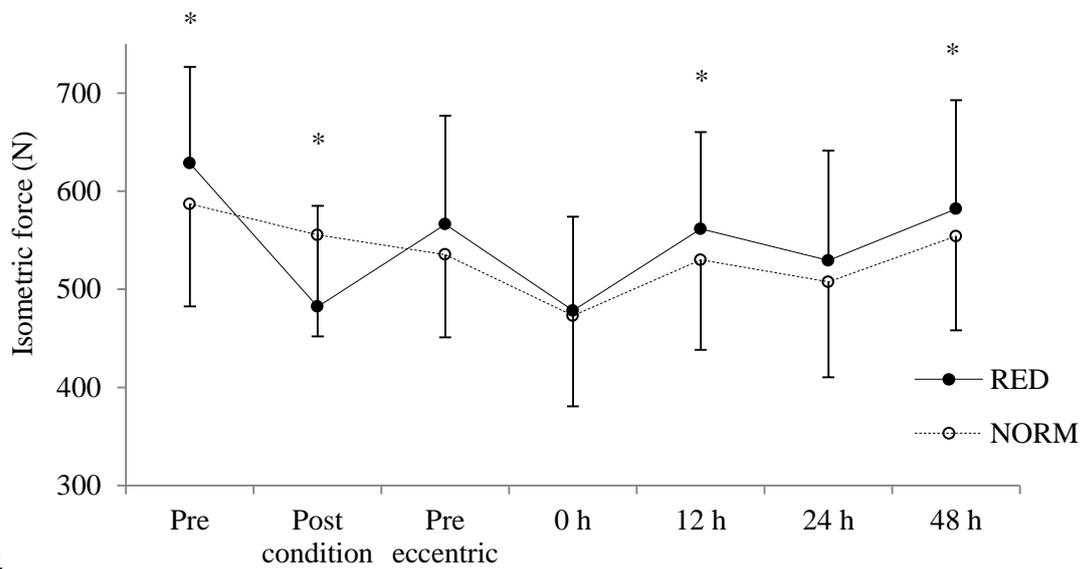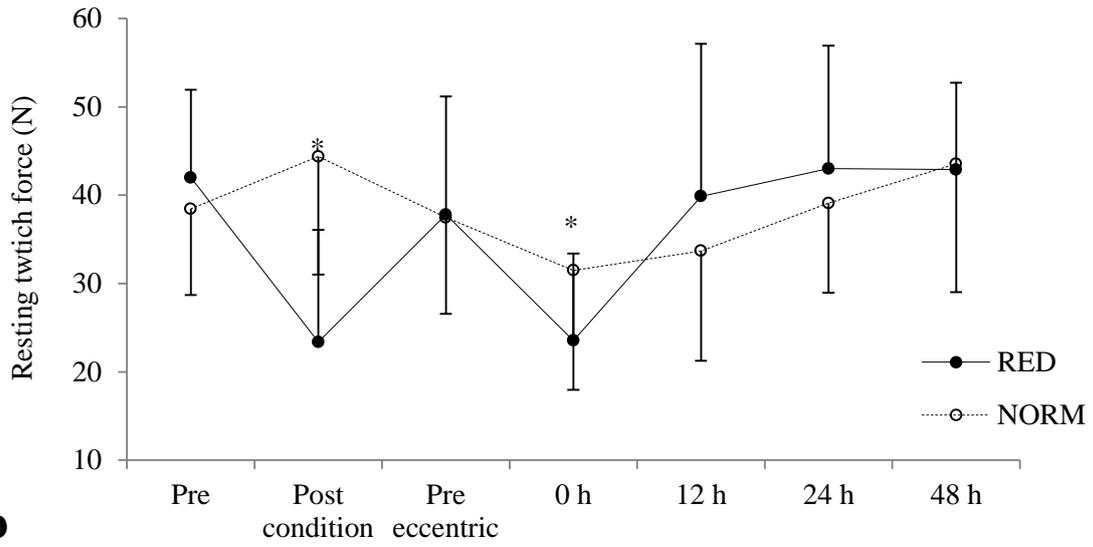
Fig. 4. Isometric maximal voluntary contraction (MVC) force (a), twitch force (b), 20:50 Hz force ratio (c) and muscle soreness (d) following maximal eccentric knee extensions with reduced (RED) and normal (NORM) glycogen availability legs. Data presented are mean±SD. * Significant difference between conditions, $P < 0.05$.