Effect of eccentric exercise with reduced muscle glycogen on plasma interleukin-6 and neuromuscular responses of *m. quadriceps femoris*

James P. Gavin, Stephen D. Myers, Mark E.T. Willems

University of Chichester
Department of Sport and Exercise Sciences
College Lane
Chichester
PO19 6PE
United Kingdom

Running head: Muscle-damaging exercise with low glycogen

Corresponding author: Prof Mark Willems
Department of Sport and Exercise Sciences
University of Chichester
College Lane
Chichester
PO19 6PE
United Kingdom

Abstract

Eccentric exercise can result in muscle damage and interleukin-6 (IL-6) secretion. Glycogen availability is a potent stimulator of IL-6 secretion. We examined effects of eccentric exercise in a low glycogen state on neuromuscular function and plasma IL-6 secretion. Twelve active males (23 ± 4 years, 179 ± 5 cm, 77 ± 10 kg) completed two downhill treadmill runs (gradient, -12%, 5x8 min; speed, 12.1 ± 1.1 $km \cdot h^{-1}$) with normal (NG) and reduced muscle glycogen (RG) in randomized order and at least six weeks apart. Muscle glycogen was reduced using an established cycling protocol until exhaustion and dietary manipulation the evening before the morning run. Physiological responses were measured up to 48 h after the downhill runs. During recovery, force deficits of *m. quadriceps femoris* by maximal isometric contractions were similar. Changes in low-frequency fatigue were larger with RG. Voluntary activation and plasma IL-6 levels were similar in recovery between conditions. It is concluded that unaccustomed, damaging eccentric exercise with low muscle glycogen of the *m. quadriceps femoris*: i) exacerbated low-frequency fatigue, but ii) had no additional effect on IL-6 secretion. Neuromuscular impairment after eccentric exercise with low muscle glycogen appears to have a greater peripheral component in early recovery.

Keyword: interleukin-6, eccentric exercise, muscle glycogen

New & Noteworthy

Athletes may perform muscle damaging exercise as part of training routines. Muscle-damaging eccentric exercise initiated with low muscle glycogen does not seem to exacerbate substantially the functional responses. In fact, voluntary force production and voluntary activation were not affected. In addition, muscle-damaging eccentric exercise with low muscle glycogen does not result in enhanced interleukin-6 levels.

Introduction

Exercise-induced muscle damage from unaccustomed eccentric contractions is characterized by myofibrillar disruption, insulin resistance (1, 33), muscle soreness (28), neuromuscular dysfunction (16, 70), and inflammation (55). Such indicators of muscle damage can be observed immediately after the exercise or with a delayed response, however, the occurrence of unaccustomed intense eccentric contractions is not necessarily required. Neuromuscular dysfunction, for example, is common after prolonged, submaximal exercise involving primarily concentric contractions, such as level running. Level running is partly maintained by the breakdown of muscle glycogen with the glycogen depletion rate related to the intensity of the exercise (22). In addition, the performance of prolonged exercise is known to be associated with glycogen availability (4). During the recovery from prolonged exercise, the replenishment of glycogen stores is required to restore neuromuscular function, and is of particular importance particularly when the performance is repeated over days (15). After concentric exercise, the replenishment of muscle glycogen is completed around 48 h later (59), whereas it can take over 10 days following eccentrically-biased, damaging exercise (50) due to the transient insulin resistance. Therefore, exercise repeated over days (i.e. athletic training) may be undertaken with incomplete glycogen replenishment. Although the effects of glycogen availability has been established for exercise involving non-damaging contractions, it is not known whether indicators of muscle damage from eccentric contractions such as neuromuscular dysfunction and markers of inflammation would be affected by glycogen availability.

Many studies have reported on the inflammatory exercise response, with different exercise models, and quantified this by an increase in interleukin-6 (IL-6). An increase in interleukin-6 was shown following muscle-damaging eccentric exercise (39), although this

has not been a consistent observation (31). Increases in IL-6 are also common after non-damaging exercise and are produced primarily within skeletal muscle (53), but also in the central nervous system (49) and ligamentous tissue (35): concentrations of IL-6 can increase up to 100-fold (57). It is thought that IL-6 may work as an energy sensor with local and systemic effects (58). Furthermore, the IL-6 response is related to exercise intensity (25, 37), duration (67), glucose availability during exercise (9, 10, 18), and glycogen availability (57).

Exercise with low muscle glycogen is known to affect metabolic pathways (21, 27, 36) and may be linked with transcriptional control of exercise-responsive genes (60, 65). With glycogen availability to be closely linked to neuromuscular fatigue and a potent stimulator of the IL-6 response with concentric exercise, the neuromuscular and plasma IL-6 responses for eccentric exercise with reduced muscle glycogen have not been examined. An augmented IL-6 response following eccentric exercise may provide a metabolic signal to meet the substrate demand of damaged muscle to enhance recovery.

The aim of the present investigation was to examine the neuromuscular dysfunction, muscle soreness and plasma IL-6 response after muscle damaging eccentric muscle with reduced muscle glycogen. It was hypothesized that muscle-damaging eccentric exercise with reduced muscle glycogen would result in larger neuromuscular dysfunction and higher plasma IL-6 levels.

Method

Participants

Twelve physically active males (age 23 ± 4 years, height 179 ± 5 cm, body mass 77 ± 10 kg, body fat 14.4 ± 3.8%, $\dot{V}O_{2max}$ 54 ± 9 mL·kg·min^{-1}, mean ± SD) provided written informed consent for study participation. Participants refrained from resistance training five

days prior to each evening pre-testing visit until 48 h following downhill running, were free from musculoskeletal injury, had no history of joint problems and were instructed not to use anti-inflammatory methods. Participants scored 3.9 ± 0.9 on the Pittsburgh Sleep Quality Index (8) indicating good sleep quality and no confounding effect on IL-6 levels (62). Approval for the study was obtained from the University of Chichester Ethics Committee.

Experimental Design

Neuromuscular function, muscle soreness, blood glucose, blood lactate and plasma IL-6 responses were measured after eccentric exercise under two conditions 1) downhill running with normal glycogen (NG) and 2) downhill running with reduced glycogen (RG)] for up to 48 h. A schematic of the experimental procedures is presented in Figure 1. The end of the downhill run (i.e. 0 h-post) is selected as the zero time point with 14 h-pre baseline measurements before the cycling protocol and 12 h-pre measurements after the cycling protocol (Figure 1). Participants visited the laboratory for two pre-testing visits and five experimental visits for each of the NG and RG conditions, respectively. For the 1st experimental visit of each condition, participants arrived in the evening before the 2nd visit the next day to perform the glycogen-reducing cycling protocol or the control condition, i.e. a seated rest on the cycle ergometer. The following morning, participants completed the downhill treadmill running protocol; with visits three to five at 12, 24 and 48 h after downhill running (i.e. 12 h-post, 24 h-post and 48 h-post, respectively). Neuromuscular responses were also measured immediately before and following the glycogen-reducing cycling protocol or control (i.e. 14 h-pre and 12 h-pre, respectively) and before and after downhill running (i.e. 1 h-pre, 0 h-post, 12 h-post, 24 h-post and 48 h-post) (Figure 1). Downhill running conditions were completed at least six weeks apart in randomized order. Blind-selection was used to randomly allocate run order (normal glycogen condition first, n = 5; reduced glycogen

condition first, *n* = 7). Participants were instructed to arrive hydrated, and not to have performed strenuous physical activity in the 24 h before each visit. For the evening session with the glycogen-reducing cycling protocol, participants were advised to consume a light meal consumed 3 h before, and instructed to consume no caffeine for the 12 h before. Thereafter, participants fasted until after the downhill running the next morning but water could be taken *ad libitum*. Participants self-recorded their habitual food intake in the 48 h preceding, and following the first downhill running condition. The self-recorded food intake was then prescribed for the subsequent condition. Each experimental session started with an explanation of the experimental procedures, followed by blood sampling, muscle soreness measurement and neuromuscular testing. Anthropometric characteristics were determined in the first pre-testing visit. Time of arrival for evening and morning sessions of the first condition were replicated for the second condition.

Pre-testing Sessions

In the first visit, height, body mass and skinfolds (Harpenden Skinfold Callipers, Baty Int., West Sussex, UK) for determination of body fat percentage (29, 74) were measured. Subsequently, participants completed a maximal incremental cycling protocol to establish the intensity for the glycogen-reducing cycling protocol. Using an electronically braked, computer programmed ergometer (Excalibur Sport 925900, Lode, Groningen, the Netherlands), participants maintained a ~75 rpm cadence at 50 W for 3 min with 10 W increments every 20 s until volitional exhaustion (51). Expired air was analysed breath-by-breath using a portable metabolic cart (Cosmed K4b^2, Rome, Italy) to establish maximum oxygen uptake (i.e. $\dot{V}O_{2max}$). At least 48 h later, in the second visit, participants completed a submaximal incremental running protocol to determine the running speed for the downhill

treadmill running protocol. For warm up, participants ran for 5 min at 8 km·h^{-1} (1% gradient) on a pre-calibrated powered treadmill (Pulsar, h/p/Cosmos Sports & Medical GmbH, Germany). Starting speed for the running protocol was 8 km·h^{-1} (1% gradient) with increments of 1 km·h^{-1} every 4 min, until eight stages were completed or volitional exhaustion was reached. Fingertip capillary blood samples were taken in the final 30 s of each stage into EDTA-coated microvettes (Sarstedt Aktiengesellschaft & Co., Nümbrecht, Germany) and analysed for blood lactate (2300 STAT Plus™ analyser, YSI Life Sciences, Yellow Springs, USA). Lactate analysis software (46) was used to calculate the running speed at the lactate threshold, which was used for the downhill running conditions.

Glycogen-Reducing Cycling Protocol

The protocol was modified from procedures used by Thomson et al. (80) and Osborne and Schneider (51). Thomson et al. (80) used for the glycogen reducing cycling a 60 rpm cadence, and muscle biopsy to establish glycogen levels in healthy males. Participants visited the laboratory in the evening (between 19:05 and 19:50 h) for the glycogen-reducing cycling protocol, 3 h after a light meal. Participants cycled at ~75 rpm for 10 min at 50% $\dot{V}O_{2max}$ to warm up; then at 60% $\dot{V}O_{2max}$ until volitional exhaustion, determined by an inability to maintain a cadence above 50 rpm. Cycling at this intensity until volitional exhaustion depleted total muscle glycogen by 77% and type I fiber glycogen by 95% (78). Mean time-to-exhaustion in the present study was 95 ± 13 min.

Downhill Running

The morning after the evening glycogen-reducing cycling protocol, participants ran downhill (-12% gradient) at their individual level running lactate threshold speed (12.1 ± 1.1

172 km·h^{-1}). Participant running speed, footwear and start time (± 5 min) were the same for both
173 conditions. Five, 8 min stages were performed, each separated by 2 min rest intervals of level
174 jogging (1% gradient; 8 km·h^{-1}) (17). Downhill runs (NG and RG) were performed in a
175 temperature controlled laboratory (~20°C).
176
177 **Neuromuscular Function**
178 ***Maximal Isometric Force***
179 Participants were seated with the hip and knee in 90° flexion, and secured at the chest
180 and waist. The right ankle was connected proximally at the fibular notch and medial
181 malleolus with a steel chain to a calibrated s-beam load-cell (RS 250 kg, Tedea Huntleigh,
182 Cardiff, UK). Force exerted by the *m. quadriceps femoris* was sampled at 1000 Hz and
183 displayed (Chart 4, v4.1.2, AD Instruments, Oxford, UK) on a desktop computer screen in
184 front of the subjects. Neuromuscular responses of *m. quadriceps femoris* were recorded from
185 the right leg with participants seated on a custom-made chair. Neuromuscular procedures
186 began with a warm up consisting of three, ~5 s submaximal contractions (i.e. 50% of
187 maximal voluntary isometric contraction [iMVC]). Subsequently, isometric strength of the *m.*
188 *quadriceps femoris* was recorded with participants producing three maximal voluntary
189 isometric contractions (iMVC) of about 3-5 seconds (with superimposed doublet, see below
190 for more details on doublet stimulation) with verbal encouragement and visual feedback
191 provided by the investigator. Rest period between iMVCs was 2-min. When there was more
192 than 10% difference between the contractions producing the highest and lowest isometric
193 contraction, further attempts were permitted. Maximal isometric force was calculated as the
194 highest mean force value over a 0.5 s period of the contraction.
195
196 ***Doublet Stimulation***

Percutaneous electrical stimulation was delivered with a DS7A electrical stimulator controlled with a NeuroLog pulse generator (Digitimer Ltd, Welwyn Garden City, UK) using two saline soaked electrodes (9 x 18 cm), positioned in the proximal and distal part of the upper leg. The position of the electrodes was marked to ensure identical placement in subsequent visit. Participants were familiarized for the neuromuscular testing procedures. Determination of maximal twitch force was initiated with 100 mA current, after which, 50 mA increments were administered until further increase resulted in no change in twitch force. To confirm maximal current, the current was then increased by a further 10%. The submaximal stimulation level represented the current that evoked a twitch force equivalent to 5% of an individual's iMVC force. Procedures for maximal and submaximal twitch assessment were determined for the NG and RG condition; within each condition, stimulation currents were established at the beginning of the evening session (submaximal current, NG: 113.7 ± 13.2 mA; RG: 116.9 ± 16.5 mA), then referred to up until the final, 48 h-post time-point. Doublet stimulation was delivered ~1 s before (resting), during the iMVC plateau (superimposed) and ~1 s following contraction (potentiated). Voluntary activation for indication of central fatigue was determined according to the resting (VA_R) and potentiated (VA_P) doublet (61). Percent voluntary activation was calculated as follows:

$$VA_R\ (\%) = [1 - (\text{superimposed doublet} \times (T_b/\text{iMVC}) \times \text{resting doublet})] \times 100\% \quad (1)$$

$$VA_P\ (\%) = [1 - (\text{superimposed doublet} \times (T_b/\text{iMVC}) \times \text{potentiated doublet})] \times 100\% \quad (2)$$

When the superimposed doublet occurred prior to, or preceding the voluntary peak force, the correction technique of Strojnik and Komi (77) was used: T_b = force immediately before or after superimposed doublet. Superimposed doublet amplitude was reassessed, according to

force immediately preceding the superimposition, in addition to the peak force value. This calculation assumes a linear relation between peak force and superimposed doublet. The original equation is corrected when T_b lies within 96% of maximal contraction force (42, 77). Rejection criteria for the doublet stimulation were: force traces displaying no clear plateau before the superimposed doublet; a superimposed doublet administered when force was not at, or close to, maximum; when the volunteer perceived their effort as submaximal when receiving doublet stimulation (73).

Low-frequency Fatigue

The neuromuscular protocol concluded with 20 and 50 Hz stimulations at rest. The submaximal twitch current, as described above under doublet stimulation (normal condition, 113.7 ± 13.2 mA; reduced condition, 116.9 ± 16.5 mA) was delivered over 0.5 s as 20 and 50 Hz stimulations to the right *m. quadriceps femoris*. Stimulations were repeated and administered in a random order [coefficient of variation (CV) ranged across time-points from: 1.1% to 3.2% for 20 Hz, and 1.0% to 2.1% for 50 Hz tetani]. Force responses for each frequency were averaged, and the low-to-high frequency force (20:50 Hz) ratio was calculated. A decrease in the ratio indicates the presence of low-frequency fatigue (LFF) (30).

Perceived Muscle Soreness

Perceived muscle soreness was assessed using a visual analog scale, ranging from 0 (no pain) to 10 (extreme pain). Soreness of the *m. quadriceps femoris* was determined prior to maximal strength by the same investigator: at rest, during passive stretch, and during voluntary contraction (40) for each experimental time-point. Each method was performed seated on the strength-testing chair, and involved verbal instruction and presentation of the visual scale. For palpation, the investigator exerted enough pressure over the mid-portion of

247 the muscle group to cause blanching under the fingernail (26). Passive stretch involved the
248 investigator manually moving the leg from ~90° knee flexion, towards ~0° flexion, until the
249 participant expressed they could no longer tolerate movement. Voluntary contraction
250 involved contracting the *m. quadriceps femoris* from ~90° knee flexion to full extension
251 (knee aligned horizontally with the ankle) over a 3 s period.
252
253 ***Plasma Interleukin-6***
254 Blood was drawn from the antecubital vein using a Precision Glide needle™ into a
255 3.0 mL EDTA-treated tube (BD Vacutainer®, Franklin Lakes, New Jersey, USA). The
256 sample was then centrifuged for 15 min at 1,000 g within 10 min of collection (Centurion
257 Scientific Ltd, Stoughton, West Sussex). Plasma was then aliquoted into a 3.0 mL
258 polystyrene no-anticoagulant tube (International Scientific Supplies Ltd, Bradford, West
259 Yorkshire) and stored at -20°C for further IL-6 analysis. Plasma was analysed in duplicate
260 using IL-6 Quantikine high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits
261 according to recommended instructions (R&D Systems Europe Ltd, Abingdon, UK). Plates
262 coated with mouse IL-6 monoclonal antibody were prepared with assay diluent, before the
263 addition of samples or standards. Assay incubations were performed at room temperature on
264 a horizontal orbital microplate shaker (Mikroshaker 20T, Camlab Ltd, UK) at 500 rpm. After
265 a 2 h incubation period, plates were washed and 200 µL IL-6 conjugate antibody was added
266 to individual wells. After 2 h, plates were washed, then the following procedure concluded:
267 addition of 50 µL substrate solution, 1 h benchtop incubation, addition of 50 µL amplifier
268 solution, 30 min benchtop incubation, and then addition of 50 µL stop solution. Within 5 min,
269 the optical density of each well was read with a TECAN GeNios microplate reader (TECAN,
270 Reading, UK) using 490 nm absorbance, and 650 nm correction wavelengths. Plasma IL-6
271 concentration was determined by plotting standard concentration data points (concentration

against corrected reading), before applying a four-parameter logistic fit. Detection limits were 0.7 (lower) and 300 pg·mL^{-1} (upper). Intra- and inter-assay CV were 4.8% and 5.4%, respectively.

Data Analysis

The effect of glycogen reduction was examined using a two-way repeated measures ANOVA to detect change from baseline values for each variable; pre-planned paired samples t-tests were subsequently used to detect significant difference between conditions (normal and reduced glycogen) and time-points [pre cycling (14 h-pre), post cycling or rest (12 h-pre), pre downhill running (1 h-pre), and after downhill running (0 h-post, 12 h-post, 24 h-post and 48 h-post)]. Greenhouse-Geisser correction was applied where assumptions of sphericity were violated. Pearson's correlation coefficients were calculated for: i) downhill running speed and immediate IL-6 concentration, and ii) downhill running end blood lactate and plasma IL-6 concentrations. Cohen's effect size was calculated with values interpreted as 0.2 for small, 0.5 for moderate, and 0.8 for large differences (79). Statistical significance was accepted at $P<0.05$. Interpretation of $0.05>P \leq 0.1$ was according to guidelines by Curran-Everett & Benos (13). Data are presented as mean ± SD. Statistical analyses were conducted using IBM SPSS Statistics, version 20 (IBM Corp, Armonk, NY).

Results

Glycogen-reducing Cycling Protocol

Blood glucose was decreased by 32% from 4.7 ± 0.4 to 3.2 ± 0.5 mmol·L^{-1} ($P < 0.01$, d = -3.31) and lactate increased by 66% from 0.87 ± 0.2 to 2.5 ± 0.8 mmol·L^{-1} ($P < 0.01$, d = 2.81) after the glycogen reduction cycling protocol. Before downhill running the next

morning, glucose was still reduced at 3.6 ± 0.4 mmol·L^{-1} (P < 0.01, *d* = -2.75) and lactate had returned to baseline (0.83 ± 0.3 mmol·L^{-1}, P = 0.6, *d* = -0.12).

In the control condition, there was a trend for glucose to decrease from 4.3 ± 0.6 to 3.9 ± 0.2 mmol·L^{-1} (P = 0.1, *d* = -0.89) with lactate slightly elevated from 0.77 ± 0.2 to 0.97 ± 0.3 mmol·L^{-1} (P = 0.03, *d* = 0.78), with both returned to baseline the following morning (glucose: 4.1 ± 0.6 mmol·L^{-1}, P = 0.7, *d* = -0.33; lactate: 0.71 ± 0.2 mmol·L^{-1}, P = 0.3, *d* = -0.30).

Maximal Isometric Force

Downhill running bouts were separated by at least 6 weeks; there was no evidence of an order effect for post-exercise force loss immediately after the run (run 1: -19 ± 1%, run 2: -15 ± 1.8% , P = 0.43) and muscle soreness (run 1: 3.6 ± 1.6, run 2: 4.5 ± 2.0 , P = 0.12). Glycogen reduction by cycling had an effect ($F_{(1,11)}$ = 20.6, P < 0.01) on maximal isometric force of the *m. quadriceps femoris*. Maximal isometric force was decreased immediately after the glycogen reduction cycling protocol by 20.8% (from 647 ± 112 N to 512 ± 95 N, P < 0.01, *d* = -1.24) with no changes in the control condition. Following downhill running, maximal isometric force was decreased by 24.6% (from 593 ± 98 N to 475 ± 99 N, P < 0.01, *d* = -1.20) and 27.7% (from 564 ± 114 N to 468 ± 93 N, P < 0.01, *d* = -0.93) for normal and reduced glycogen conditions (Figure 2), respectively. In the normal glycogen condition, maximal isometric force was still decreased at 12 h-post (527 ± 106 N, 16.6%, P = 0.02, *d* = -0.96), 24 h-post (540 ± 109 N, 14.5%, P = 0.01, *d* = -0.83) and 48 h-post (556 ± 105 N 12.1%, P = 0.01, *d* = -0.71). For the reduced glycogen condition, maximal isometric force remained decreased at 12 h-post (537 ± 89 N, 17%, P < 0.01, *d* = -1.03), 24 h-post (529 ± 100 N, 18.2%, P < 0.01, *d* = -1.06) and 48 h-post (528 ± 97 N,18.4%, P < 0.01, *d* = -1.08). At

each time-point during recovery from downhill running, maximal isometric force values were similar for both conditions.

Doublet Force Parameters

During rest, the doublet force showed a condition x time interaction ($F_{(1,11)} = 6.3$, $P < 0.01$) (Figure 3). The doublet force was reduced after the glycogen-reducing cycling protocol by 22.6% (from 84 ± 16 N to 65 ± 17 N, $P < 0.01$, $d = -1.15$) and unchanged in the control condition (80 ± 18 N and 83 ± 19 N, $P = 0.2$, $d = 0.17$). The next morning before the downhill running, the doublet force was at baseline values in both conditions (80 ± 16 N, glycogen reduced, $d = -0.28$; 80 ± 20 N, normal $d = -0.01$, both $P > 0.05$). Immediately after downhill running, the doublet force was reduced by 25.4% for the low glycogen condition (from 84 ± 16 N to 63 ± 12 N, $P < 0.01$, $d = -1.51$), and 17.6% for the control condition (from 80 ± 18 N to 66 ± 18 N, $P < 0.01$, $d = -0.77$). For the low glycogen condition, the doublet force remained decreased at 12 h-post (65 ± 17 N, 22.7%, $P < 0.01$, $d = -1.17$) and 24 h-post (66 ± 16 N, 20.9%, $P < 0.01$, $d = -1.12$), with a trend to be lower at 48 h-post (74 ± 17 N, $P = 0.1$, $d = -0.63$). In the control condition, the doublet force was reduced at 12 h-post (67 ± 20 N, 16.2%, $P < 0.01$, $d = -0.69$), 24 h-post (66 ± 21 N, 18.2%, $P < 0.01$, $d = -0.75$) and 48 h-post (63 ± 18 N, 21.5%, $P < 0.01$, $d = -0.96$). Contraction time of the doublet displayed no time ($F_{(1,11)} = 0.631$, $P = 0.70$) or condition effect ($F_{(1,9)} = 0.112$, $P = 0.75$) (Figure 4). Average rate of force development showed no time ($F_{(1,10)} = 1.156$, $P = 0.30$) or condition effect ($F_{(1,10)} = 0.117$, $P = 0.70$) (Figure 5). A time effect was seen for half-relaxation time ($F_{(1,10)} = 21.8$, $P < 0.01$), with no difference between conditions at similar time-points (Figure 6).

Voluntary Activation

There was a time ($F_{(1,10)}$ = 8.7, P < 0.01), but no condition effect ($F_{(1,10)}$ = 0.365, P = 0.5) for voluntary activation for the resting doublet (i.e. VA_R) (Figure 7). Voluntary activation for the resting doublet was decreased after the glycogen-reducing cycling protocol (from 95.8 ± 2.3% to 91.3 ± 4.2%, P < 0.01, *d* = -1.33) and was returned to baseline the next morning (P = 0.5, *d* = -0.34). After downhill running with low muscle glycogen, voluntary activation for the resting doublet was reduced at 0 h-post (90.1 ± 3.0%, *d* = -2.13), at 12 h-post (91.8 ± 2.8%, *d* = -1.56), 24 h-post (90.6 ± 2.3%, *d* = -2.26) and 48 h-post (91.2 ± 2.6%, *d* = -1.87) (all P < 0.01). In the normal condition, voluntary activation for the resting doublet was decreased at 0 h-post (from 94.9 ± 3.1% to 89.7 ± 3.5%, *d* = -1.57), at 12 h-post (90.6 ± 3.4%, *d* = -1.32), 24 h-post (90.2 ± 4.0%, *d* = -1.31) and 48 h-post (90.1 ± 3.5%, *d* = -1.45) (all P < 0.01). For voluntary activation of the potentiated doublet (i.e. VA_P), there was a time effect ($F_{(1,11)}$=16.3, P < 0.01, *d* = 0.59), but no condition effect ($F_{(1,11)}$ = 0.681, P = 0.4, *d* = 0.06, Figure 8). Voluntary activation for the potentiated doublet was decreased after the glycogen-reducing cycling protocol (from 96.2 ± 2.1% to 93.0 ± 3.0%, P < 0.01, *d* = -1.24) and returned to baseline the next morning. After downhill running with low muscle glycogen, voluntary activation for the potentiated doublet was reduced at 0 h-post (91.7 ± 2.5%, *d* = -1.95) at 12 h-post (92.5 ± 2.0%, *d* = -1.80), 24 h-post (92.0 ± 1.9%, *d* = -2.10), and 48 h-post and (92.1 ± 2.0%, *d* = -2.00) (all P < 0.05). In the normal condition after downhill running, voluntary activation for the potentiated doublet was reduced at 0 h-post (from 95.3 ± 2.5% to 91.3 ± 2.9%, *d* = -1.48), at 12 h-post (91.6 ± 3.0%, *d* = -1.34), 24 h-post (91.9 ± 3.1%, *d* = -1.21) and 48 h-post (91.1 ± 3.2%, *d* = -1.46) (all P < 0.01).

20 Hz and 50 Hz Force Responses

The force at 20 Hz stimulation displayed a significant condition x time effect ($F_{(1,11)}$=3.4, P < 0.01) (Figure 9). After the glycogen-reducing cycling protocol, the force at 20 Hz was

reduced by 17.9% (from 133.6 ± 39.5 N to 109.7 ± 29.5 N, P = 0.03, *d* = -0.69), and returned to baseline the next morning (P > 0.05, *d* = 0.20). Following downhill running with low muscle glycogen, the force at 20 Hz was reduced at 0 h-post by 18.2% (109.3 ± 26.4 N, , P < 0.01, *d* = -0.72), at 12 h-post by 18.3% (109.1 ± 35.6 N, P = 0.02, *d* = -0.65) with a trend for a decrease at 24 h-post by 16.7% (111.3 ± 36.7 N, P = 0.07, *d* = -0.58) and 48 h-post by 11.7% (118.0 ± 29.7 N, P = 0.1, *d* = -0.45). In the control condition, the force at 20 Hz was decreased at 0 h-post by 21.5% (from 129.3 ± 35.3 N to 102.8 ± 28.9 N, P = 0.01, *d* = -0.82), with a trend for a decrease at 12 h-post by 14% (111.3 ± 47.3 N, P = 0.11, *d* = -0.43), and a decrease at 48 h-post by 19.5% (104.1 ± 40.4, P = 0.02, *d* = -0.66). Between the conditions, there was only a difference in force at 20 Hz as a result of the glycogen-reducing cycling protocol (P = 0.04).

The force at 50 Hz stimulation showed a time ($F_{(1,11)}$ = 5.8, P < 0.01, *d* = 0.35), but no condition effect ($F_{(1,11)}$ = 3.8, P = 0.5, *d* = 0.03). Following downhill running with low muscle glycogen, the force at 50 Hz was reduced only at 24 h-post by 16.7% (from 168.5 ± 57.0 N to 144.4 ± 48.6 N, P = 0.05, *d* = -0.46). In the control condition, the force at 50 Hz was reduced only after 48 h-post by 19.2% (from 157.7 ± 51.1 N to 127.5 ± 54.6 N, P = 0.04, *d* = -0.57) (Figure 10).

Low-to-high frequency ratio exhibited a significant condition x time effect ($F_{(1,11)}$=17.4, P < 0.01, *d* = 0.61; Figure 11). The glycogen-reducing cycling protocol resulted in a reduced ratio by 20.3% (from 0.80 ± 0.06 to 0.60 ± 0.12, P < 0.01, *d* = -2.11). A reduced ratio was also present at 0 h-post by 15.4% (0.65 ± 0.07, P < 0.01, *d* = -2.30) and 12 h-post by 7.7% (0.73 ± 0.05, P < 0.01, *d* = -1.27) (Figure 11). Recovery for low-to-high frequency was complete by 24 h-post (P = 0.2). In the condition with normal glycogen, the ratio was decreased at 0 h-post by 10.7% (from 0.84 ± 0.10 to 0.70 ± 0.09, P < 0.01, *d* = -1.47) and 12 h-post by 3% (0.77 ± 0.07, P < 0.01, *d* = -0.81). The relative ratio decrease was substantially

greater at 0 h-post (P = 0.02, *d* = 0.57) and 48 h-post (P = 0.03, *d* = 0.84) in the reduced glycogen condition with moderate and large effects, respectively.

Perceived Muscle Soreness

Soreness of the *m. quadriceps femoris* in rest showed a significant condition x time interaction ($F_{(1,10)}$=17.9, P < 0.01, *d* = 0.64) (Figure 12). Soreness was similar at 14 h-pre, becoming greater after the glycogen-reducing cycling protocol, in comparison to normal glycogen (P = 0.01). Soreness was still elevated above normal the following morning, before (from 1.2 ± 0.4 to 2.1 ± 1.0, P = 0.05), and immediately after downhill running for the reduced glycogen condition (3.9 ± 1.8, P = 0.03). Thereafter, no difference existed between conditions.

Perceived soreness under contraction ($F_{(1,10)}$=17.2, P < 0.01, *d* = 0.63) (Figure 13) and during passive stretch (Figure 14) exhibited significant condition x time effects ($F_{(1,10)}$=12.6, P < 0.01, *d* = 0.56) with no differences between conditions during the recovery from downhill running.

Interleukin-6 Response

Plasma IL-6 concentration displayed a significant condition x time effect ($F_{(1,10)}$=24.7, P < 0.01, *d* = 0.71; Figure 15). After the glycogen-reducing cycling protocol, IL-6 was increased above baseline (from 0.69 ± 0.34 pg·mL^{-1} to 8.1 ± 3.05 pg·mL^{-1}, P < 0.01, *d* = 3.40), as well as at 0 h-post (4.49 ± 2.92 pg·mL^{-1}, P < 0.01, *d* = 1.94), 12 h-post (2.28 ± 2.0 pg·mL^{-1}, P = 0.02, *d* = 1.07), 24 h-post (1.03 ± 0.71 pg·mL^{-1}, P = 0.05, *d* = 0.73), and 48 h-post (1.10 ± 0.84 pg·mL^{-1}, P = 0.04, *d* = 0.76). In the normal glycogen condition, IL-6 was elevated at 0 h-post (from 0.59 ± 0.21 pg·mL^{-1} to 4.32 ± 3.17 pg·mL^{-1}, P < 0.01, *d* = 1.66) and at 12 h-post

(2.01 ± 1.64 pg·mL^{-1}, P = 0.02, *d* = 1.22). There were no differences between IL-6 values at similar time-points during recovery from downhill running in both conditions.

No significant correlation was observed for downhill running speed and immediate IL-6 response for normal (*r* = -0.03, P = 0.4) and glycogen-reduced condition (*r* = 0.30, P = 0.3). No significant correlation was observed between blood lactate at the end of the downhill run and immediate IL-6 response in normal (*r* = -0.78, P = 0.4) and reduced glycogen condition (*r* = 0.46, P = 0.14).

Discussion

The main finding of the present study was that the change in the low-to-high frequency force ratio after muscle-damaging eccentric exercise was greater when the exercise was performed with low muscle glycogen. Aside from greater muscle soreness in rest immediately after downhill running with reduced glycogen, there were no differences in voluntary strength, doublet force, muscle soreness, voluntary activation losses, and plasma IL-6 responses between conditions up to 48 h later. These findings suggest that impaired neuromuscular function following muscle-damaging exercise with reduced muscle glycogen is derived predominantly from peripheral mechanisms. That no condition effect was seen for plasma IL-6 levels, and doublet responses at 24 h-post and 48 h-post after eccentric exercise may indicate partial muscle glycogen recovery.

As far as we know, this is the first study to examine the effect of low muscle glycogen during muscle-damaging eccentric exercise on the IL-6 response and central and peripheral fatigue mechanisms. During prolonged, submaximal exercise, glycogen availability becomes critical to maintain performance (24). We used an established cycling protocol, shown to result in significant lowering of muscle glycogen as quantified with muscle biopsy (80), to investigate whether neuromuscular and IL-6 responses would be altered for a subsequent bout

444 of damaging, eccentric exercise. Glycogen reduction was substantiated by an inability to
445 sustain workload during preliminary cycling, decreased blood glucose (-31.9%) and
446 voluntary activation of the *m. quadriceps femoris* (-4.5%) after the cycling exercise, in
447 addition to elevated blood lactate (2.5 $mmol \cdot L^{-1}$). Responses to glycogen reduction cycling
448 were in line with earlier work examining prolonged cycling on neuromuscular fatigue (38).
449 Glycogen reduction cycling induced a -20.8% decrement in maximal voluntary force
450 production, alongside voluntary activation losses (-4.5%, VA_R, -3.2%, VA_P). The exhaustive
451 cycling also caused significant depression in resting doublet response (-22.6%) and low-to-
452 high frequency force ratio (-20.3%). Half-relaxation time was also shorter (-23%) and muscle
453 soreness slightly elevated (1.3) immediately after the cycling. Cycling exercise involves the
454 *m. quadriceps femoris* performing, primarily, concentric contractions, and consequently
455 elicits less muscle damage than running exercise. Prior to eccentric exercise, neuromuscular
456 function, IL-6 and blood lactate levels were fully recovered; slight changes were seen for
457 decreased maximal voluntary force and increased tenderness. Downhill running induced
458 similar immediate maximal voluntary force losses in normal (-24.6%), and reduced glycogen
459 (-27.7%) conditions. Accompanied reductions in doublet response of -14.1% for normal
460 glycogen, and -25.4% for the glycogen reduction condition, demonstrate decreased
461 contractile excitation with reduced muscle glycogen. Impaired neuromuscular propagation for
462 the reduced glycogen condition may be explained by the additive effect of i) an initial failure
463 in Ca^{2+} release after exhaustive cycling and, ii) an elevated intracellular Ca^{2+} concentration
464 due to exercise-induced ultrastructural damage after downhill running. Disturbed Ca^{2+}
465 homeostasis is known to arise from reduced muscle glycogen concentration. Chin and Allen
466 (11) stimulated rat skeletal muscle with repeated tetani reducing glycogen content to ~25%.
467 Lowered muscle glycogen was associated with diminished force, Ca^{2+} release, and fatigue
468 attributed to excitation-contraction (E-C) coupling failure. It was purported that with depleted

glycogen and PCr, ATP concentration may temporarily decrease prior to declines in force production and Ca^{2+} release. Decreased ATP levels would, therefore, disrupt E-C coupling. More recently, Green and co-workers (23) identified close association between Ca^{2+} release and fatigue induced by 2 h cycling exercise at 62% $\dot{V}O_{2peak}$. Consistent with Chin and Allen's (11) observation, was a greater decrease in low-to-high frequency force ratio following exhaustive cycling, and then after eccentric exercise up to 24 h-post in the glycogen reduced condition. However, similar to Booth and colleagues (5), we observed decreased maximal force of the *m. quadriceps femoris*, but faster muscle relaxation time after prolonged, exhaustive cycling. These authors induced fatigue with 75% $\dot{V}O_{2peak}$ cycling (time-to-exhaustion, 72 ± 4 min) which caused 90% glycogen depletion, lowered Ca^{2+} uptake and twitch response depressed by -45% in untrained males. Fatigue is normally attended by maximal force loss and slower muscle relaxation due to impaired Ca^{2+} uptake. Booth et al. (5) proposed that the dissociation between reduced Ca^{2+} uptake and slowed muscle relaxation could be explained by exercise intensity. Higher cycling intensities may have reduced Ca^{2+} uptake to a critical threshold, leading to slower relaxation time. Our findings of faster half-relaxation times after glycogen reduction cycling, and eccentric exercise in both conditions may be more associated to intensity and damage, as opposed to rise intramuscular temperature. That half-relaxation rate was accelerated in the 12 h following eccentric exercise could potentially relate to a transient increased cross-bridge detachment, since the *m. quadriceps femoris* did not perform any external, dynamic work during post-eccentric measures. Supporting Chin and Allen's (11) assertion, fatiguing exercise decreased muscle glycogen, in addition to ATP (-20%) and PCr (-58%). We observed near recovery of neuromuscular function for the glycogen reduced condition prior to downhill running. Yet, as

492 blood glucose remained lower and tenderness higher from the onset of damaging exercise, no
493 doubt cellular and metabolic disturbances remained (23).
494 Substantial mechanical stress incurred by eccentric exercise presents ultrastructural
495 disruption, leading to reduced sarcoplasmic reticulum Ca^{2+} release and elevated sarcoplasmic
496 Ca^{2+} concentration, thus impairing E-C coupling processes (12, 64). Eccentric exercise is
497 known to induce morphological damage and E-C uncoupling in animals (78) and humans
498 (47). Upon completion of damaging exercise, we observed greater impairment in doublet
499 response for the glycogen reduced condition (normal, -14.1%; reduced, -25.4%).
500 Interestingly, where doublet force was recovered by 48 h in the reduced glycogen condition,
501 it was still significantly depressed for the normal glycogen condition (-21.5%). There was a
502 non-significant trend towards higher force loss in the reduced glycogen condition at 12 h-post
503 (normal, -16.2%; reduced, -22.7%) and 24 h-post (normal, -18.2%; reduced, -20.9%). These
504 data would suggest a greater, immediate decline in neuromuscular propagation, with faster
505 recovery in the glycogen reduced condition. Similar response was seen for the 20 Hz force,
506 but not for maximal voluntary strength or voluntary activation indices. Under normal
507 conditions, consistent doublet force loss throughout recovery was due to muscle damage.
508 Under reduced glycogen conditions, greater transient doublet loss may be due to impaired
509 Ca^{2+} release disrupting E-C coupling processes. The flux between performing: fatiguing,
510 concentric exercise that transiently lowers Ca^{2+} release; and then damaging, eccentric
511 exercise that may elevate intramuscular Ca^{2+} concentration may mediate the rapid recovery
512 for reduced glycogen state. Commencing eccentric exercise with lowered intramuscular Ca^{2+}
513 levels would not prevent damage, but may 'offset' Ca^{2+} accumulation seen under normal
514 conditions.
515 Another mechanism for the depressed doublet response is a rightward shift in the length-
516 tension relationship of the *m. quadriceps femoris*. This shift would change optimum force

production to longer muscle lengths (63). For the same joint angle, doublet force would, therefore, be underestimated. However, as length-tension properties are mechanical, and not metabolic, lowered muscle glycogen would likely have minimal influence.

Low-frequency fatigue (LFF) arises due to disrupted E-C coupling via Ca^{2+} induced ultrastructural damage (11, 82). LFF of the *m. quadriceps femoris* is common after eccentric (41, 68, 81) and endurance exercise (44, 45). To date, no studies have examined the force-frequency characteristics of the *m. quadriceps femoris* after eccentric exercise preceded by prolonged, exhaustive exercise. We observed a decreased 20 Hz response after eccentric exercise in the glycogen reduced condition up until 12 h-post, after which a trend was displayed (-16.7%, 24 h-post; -11.7%, 48 h-post). Low frequency force was similarly depressed under normal conditions, albeit still significantly 48 h-post. These temporal responses are comparable to those previously discussed for the doublet response, further evidencing a peripheral component with reduced glycogen. LFF was greater immediately after eccentric exercise with reduced glycogen (-15.4% loss), than with normal glycogen (-10.7%). Post 12 h, LFF was present for the reduced (-7.7%), and less so for the normal glycogen condition (-3%). Thereafter, LFF was non-significantly decreased for the glycogen-reduced condition (-2.8%, 24 h-post; -2.7%, 48 h-post), but not for the normal glycogen condition (1.2%, 24 h-post; 2.6%, 48 h-post). Marginal decreases in LFF with reduced glycogen, indicates near recovery one day after downhill running, but LFF remained two days later. For normal glycogen, LFF appeared less severe and subsided sooner in this cohort. Our data is not dissimilar to low-to-high frequency alterations immediately (~-17%) and 30 min (~-10%) after 30 min downhill running with active males (41). Our LFF data, again, may relate to differences in intramuscular Ca^{2+} sensitivity, with decreased tetanic Ca^{2+} with reduced glycogen damage. Therefore, muscle glycogen availability seems to enhance the recovery E-C coupling processes after exercise-induced muscle damage. Symptoms of LFF

have been documented in the plantarflexors after 5 h cross-country running in trained males (20). Although not ultra-endurance distance, it is likely our downhill protocol fatigued lower leg musculature as our cohort consisted of untrained, participants unaccustomed to repeated stretch-shortening cycles and ground-reaction forces. Greater negative work is done by the plantarflexors during downhill running, in comparison to flat running (6). Glycogen reduction cycling did not induce long-lasting fatigue upon the knee extensors. However, as the plantarflexors perform more work from early stance during downhill motion (34), if pre-fatigued, altered kinematics may induce different neuromuscular fatigue of the *m. quadriceps femoris*.

Our voluntary activation observations document persistence of central fatigue, irrespective of muscle glycogen state. Decreased neural drive to active muscle is a suggested neuromuscular mechanism limiting further trauma to the muscle-tendon unit (66). Attendant muscle tenderness may indicate group III and IV muscle afferent activity, which can modulate neuromuscular function at the spinal (2) and/or motor cortex level (43). Recently, Behrens and co-workers (3) failed to find association between voluntary activation and muscle soreness in the 72 h recovery from maximal eccentric knee extensions. However, early force loss influenced by decreased voluntary activation and impaired contractile parameters, whereas prolonged force loss was attributed to altered contractile parameters. We observed similar voluntary activation losses for normal and reduced glycogen conditions, which remained decreased 48 h after the eccentric exercise. Where the doublet response and low-to-high frequency force recover within 24 h, voluntary activation showed little recovery two days later. Voluntary activation decrease was greatest immediately after eccentric exercise. Previously, Millet et al. (45) reported a -7.6% loss in voluntary activation of the *m. quadriceps femoris* following a 30 km run. Although more severe, a long-distance run would be expected to cause both glycogen depletion and muscle damage, albeit over a longer

duration. Burnley and associates (7) recently described a critical threshold for the onset of knee extensor neuromuscular fatigue. Findings from isometric submaximal contractions have implications for downhill running; particularly the transition from low-to-high intensity contractions, as consequence of neuromuscular impairment, and therefore increased force requirement. Divergent neuromuscular fatigue profiles were suggested, according to contraction intensity, upon which reduced glycogen may impose greater metabolic stress, and therefore unique neuromuscular damage.

The emergence of muscle soreness was not concordant with the peak inflammatory response represented by IL-6. Soreness peaked between 24 h-post and 48 h-post for both conditions, whereas the highest IL-6 levels were seen post glycogen reduction cycling and immediately after eccentric exercise. In addition to prolonged IL-6 recovery, these indicate a rapid inflammatory response determined by muscle contraction and ultrastructural damage. Earlier, Deschenes et al. (14) found decreased knee extensor neuromuscular efficiency more persistent than elevations in IL-1β and creatine kinase following a bout of maximal eccentric contractions. These authors reported greatest soreness at 48 h, and a bimodal IL-1β response, with peaks at 24 h and seven days. We may have observed similar with an extended recovery period, however this is unlikely given that IL-6 concentrations were 1.1±0.8 $pg \cdot mL^{-1}$ and 1.18±0.6 $pg \cdot mL^{-1}$ at 48 h-post for reduced and normal glycogen conditions, respectively. Our IL-6 concentrations immediately following downhill running (normal, 4.32 ± 3.2 $pg \cdot mL^{-1}$; reduced, 4.49 ± 2.9 $pg \cdot mL^{-1}$) were lower than those found by Robson-Ansley et al. (69) after ~40 min flat running (6.9 ± 2.5 $pg \cdot mL^{-1}$). Running exercise is a potent stimulator of blood-borne IL-6 (19, 54). Although we both measured plasma IL-6 after treadmill running of similar durations, disparities may be explained by participant training status and exercise intensity. Robson-Ansley et al. (69) had trained runners perform a flat 10 km treadmill time-trial, whereas we had non-trained, recreationally active males completing a downhill (-12%)

592 treadmill run. Difference in energy cost for level- and downhill running is reflected in that we
593 found post-exercise lactate and glucose concentrations of 1.9 ± 0.9 mmol·L^{-1} and 4.6 ± 0.5
594 mmol·L^{-1}; whereas Robson-Ansley et al. (69) reported concentrations of 6.9 ± 2.4 mmol·L^{-1}
595 and 7.0 ± 1.9 mmol·L^{-1}. Furthermore, endurance training has been reported to attenuate IL-6
596 response following an acute bout of knee extension exercise (19, 32). Therefore, the IL-6
597 concentrations seen for downhill running may have been even lower with trained individuals.
598 However, Scott and co-workers (72) found that training status did not influence plasma IL-6
599 activity after exhaustive running. Upon volitional exhaustion following 60 min of treadmill
600 running (65% $\dot{V}O_{2max}$), with repeated bouts (70% $\dot{V}O_{2max}$), IL-6 concentration was similar
601 between untrained (29.9 ± 13.2 pg·mL^{-1}) and endurance-trained males (31.9 ± 21.5 pg·mL^{-1}).
602 Exercising to exhaustion caused a pronounced acute IL-6 response, yet concentrations had
603 returned to baseline by 24 h. Herein, increased IL-6 and decreased voluntary activation were
604 seen at 48 h post-eccentric under both conditions, providing evidence of long-lasting
605 neuromuscular and metabolic perturbations.
606 Upon commencing exercise, muscle glycogen content is a major determinant of IL-6 mRNA
607 in active muscle (19). The IL-6 response to acute, endurance exercise is stimulated by
608 increasing intensity (25, 71) and reduced muscle glycogen content (9), yet attenuated by
609 carbohydrate ingestion (48, 75). Herein, muscle glycogen state had scant effect on blood-
610 borne IL-6 response. Elsewhere similar IL-6 levels have been reported following marathon
611 running under carbohydrate-fed and control conditions (48). An explanation may be the
612 relatively low exercise intensity, and duration for our downhill protocol. Helge et al. (25)
613 reported greater IL-6 release with increasing exercise intensity and glucose uptake for the
614 knee extensors. Furthermore, work capacity was required to exceed 65% maximum to induce
615 significant knee extensor IL-6 release. Comparisons are difficult considering they adopted

616 concentric contractions in isolation, whereas as we used eccentric contractions during whole-
617 body exercise. During glycogen depletion with increasing exercise intensity, substrate
618 demand is met by hepatic glucose output. Considering that IL-6 elevates hepatic release (76),
619 and circulating IL-6 is induced by high intensity and prolonged activity, one would expect
620 enhanced glucose delivery to the exercising musculature. We observed that plasma IL-6 was
621 recovered to resting levels the morning after glycogen reducing exercise. Therefore, i)
622 preliminary exercise may not have reduced glycogen content to threshold values required to
623 induce significant IL-6 response, or ii) the intensity of the eccentric exercise may have been
624 insufficient to evoke noticeable response between conditions. With similar participants,
625 Ostapiuk-Karolczuk et al. (52) reported that muscle damage closely was associated with the
626 pro-inflammatory response after 90 min downhill running at 65% $\dot{V}O_{2max}$. Immediate IL-6
627 response was seen post-exercise, with concentrations reach peak at 6 h post. Muscle damage
628 was evidenced by creatine kinase release, yet this provides little information regarding
629 muscular performance.
630 Our study demonstrated that commencing unaccustomed, damaging exercise with lowered
631 muscle glycogen of the *m. quadriceps femoris*: i) exacerbated LFF, and ii) had no additional
632 effect on plasma IL-6 response. However, reduced muscle glycogen did not influence the
633 recovery of voluntary force loss, voluntary activation and soreness. These results indicate
634 neuromuscular impairments following muscle damaging activity derive from peripheral
635 origins with lowered glycogen availability. Evidence for peripheral neuromuscular
636 mechanisms are as follows, i) E-C coupling disturbance as reflect by decreased LFF ratio, ii)
637 depressed doublet response, and iii) similar voluntary maximal force and activation loss.
638 Therefore, initial force loss following eccentric exercise may be attributed to peripheral
639 mechanisms, particularly E-C coupling disruption with lowered muscle glycogen. Evidence

for this was seen in the prolonged recovery of low-to-high frequency force ratio. Prolonged force loss seemed to be governed by central mechanisms, independent of glycogen availability. These findings have implications for individuals performing successive bouts of exercise involving glycogen depletion, and muscle damage. From a mechanistic standpoint, our data demonstrate neuromuscular fatigue profiles following damaging exercise are influenced by muscle glycogen availability.

Acknowledgement

Dr James Gavin present address: Bournemouth University, Department of Sport and Physical Activity, Faculty of Management, Fern Barrow, Poole Dorset, BH12 5BB, United Kingdom.

References

1. **Asp S, Richter EA**. Decreased insulin action on muscle glucose transport after eccentric contractions in rats. *J Appl Physiol* 81: 1924-1928, 1996.

2. **Avela J, Finni J, Komi PV**. Excitability of the soleus reflex arc during intensive stretch-shortening cycle exercise in two power-trained athlete groups. *Eur J Appl Physiol* 97: 486-493, 2006.

3. **Behrens M, Mau-Moeller A, Bruhn S**. Effect of exercise-induced muscle damage on neuromuscular function of the quadriceps muscle. *Int J Sports Med* 3398): 600-606, 2012.

4. **Bergström J, Hermansen L, Hultman E, Saltin B**. Diet, muscle glycogen and physical performance. *Acta Physiol Scand*, 71: 140-150, 1967.

5. **Booth J, McKenna MJ, Ruell PA, Gwinn TH, Davis GM, Thompson MW**, **Harmer AR, Hunter SK, Sutton JR**. Impaired calcium pump function does not slow relaxation in human skeletal muscle after prolonged exercise. *J Appl Physiol* 83: 511-521, 1997.

6. **Buczek FL, Cavanagh PR**. Stance phase knee and ankle kinematics and kinetics during level and downhill running. *Med Sci Sports Exerc* 22: 669-677, 1990.

7. **Burnley M, Vanhatalo A, Jones AM**. Distinct profiles of neuromuscular fatigue during muscle contractions below and above the critical torque in humans. *J Appl Physiol* 113: 215-223, 2012.

8. **Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ**. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* 28: 193-213, 1989.

9. **Chan MH, Carey AL, Watt MJ, Febbraio MA**. Cytokine gene expression in human skeletal muscle during concentric contraction: evidence that IL-8, like IL-6, is influenced by glycogen availability. *Am J Physiol Regul Integr Comp Physiol* 287, R322-327, 2004a.

10. **Chan MH, McGee SL, Watt MJ, Hargreaves M, Febbraio MA**. Altering dietary nutrient intake that reduces glycogen content leads to phosphorylation of nuclear p38 MAP kinase in human skeletal muscle: association with IL-6 gene transcription during contraction. *FASEB J* 18(14): 1785-1787, 2004b.

11. **Chin ER, Allen DG**. Effects of reduced muscle glycogen concentration on force, Ca^{2+} release and contractile protein function in intact mouse skeletal muscle. *J Physiol* 498 (Pt 1): 17-29, 1997.

12. **Corona BT, Balog EM, Doyle JA, Rupp JC, Luke RC, Ingalls CP**. Junctophilin damage contributes to early strength deficits and EC coupling failure after eccentric contractions. *Am J Physiol Cell Physiol* 298: C365-376, 2010.

13. **Curran-Everett D, Benos DJ**. (2004). Guidelines for reporting statistics in journals published by the American Physiological Society. *Advanc in Physiol Edu* 28, 85-87, 2004.

14. **Deschenes MR, Brewer RE, Bush JA, McCoy RW, Volek JS, Kraemer WJ**. Neuromuscular disturbance outlasts other symptoms of exercise-induced muscle damage. *J Neurol Sci* 174: 92-99, 2000.

15. **Doyle JA, Sherman WM, Strauss RL**. Effects of eccentric and concentric exercise on muscle glycogen replenishment. *J Appl Physiol* 74: 1848-1855, 1993.

16. **Dundon JM, Cirillo J, Semmler JG**. Low-frequency fatigue and neuromuscular performance after exercise-induced damage to elbow flexor muscles. *J Appl Physiol* (1985): 105(4):1146-1155, 2008.

17. **Eston RG, Lemmey AB, McHugh P, Byrne C, Walsh SE**. Effect of stride length on symptoms of exercise-induced muscle damage during a repeated bout of downhill running. *Scand J Med Sci Sports* 10: 199-204, 2000.

18. **Febbraio MA, Steensberg A, Keller C, Starkie RL, Nielsen HB, Krustrup P. Ott P, Secher NH, Pedersen BK**. Glucose ingestion attenuates interleukin-6 release from contracting skeletal muscle in humans. *J Physiol* 549: 607-612, 2003.

19. **Fischer CP, Plomgaard P, Hansen AK, Pilegaard H, Saltin B, Pedersen BK**. Endurance training reduces the contraction-induced interleukin-6 mRNA expression in human skeletal muscle. *Am J Physiol Endocrinol Metab* 287: E1189-1194, 2004.

20. **Fourchet F, Millet, GP, Tomazin K, Guex K, Nosaka K, Edouard P, Degache F, Millet GY**. Effects of a 5-h hilly running on ankle plantar and dorsal flexor force and fatigability. *Eur J Appl Physiol* 112: 2645-2652, 2012.

21. **Gibala MJ, Peirce N, Constantin-Teodosiu D, Greenhaff PL**. Exercise with low muscle glycogen augments TCA cycle anaplerosis but impairs oxidative energy provision in humans. *J Physiol* 540(Pt 3): 1079-1086, 2002.

22. **Gollnick PD, Piehl K, Saltin B**. Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. *J Physiol* 241: 45-57, 1974.

23. **Green HJ, Duhamel TA, Smith IC, Rich SM, Thomas MM, Ouyang J, Yau JE**. Muscle fatigue and excitation-contraction coupling responses following a session of prolonged cycling. *Acta Physiol (Oxf)* 203: 441-455, 2011.

24. **Hawley JA, Schabort EJ, Noakes TD, Dennis SC**. Carbohydrate-loading and exercise performance. An update. *Sports Med* 24: 73-81, 1997.

25. **Helge JW, Stallknecht B, Pedersen BK, Galbo H, Kiens B, Richter EA**. The effect of graded exercise on IL-6 release and glucose uptake in human skeletal muscle. *J Physiol* 546: 299-305, 2003.

26. **Hickner RC, Mehta PM, Dyck D, Devita P, Houmard JA, Koves T, Byrd PL**. Relationship between fat-to-fat-free mass ratio and decrements in leg strength after downhill running. *J Appl Physiol* 90: 1334-1341, 2001.

27. **Hulston CJ, Venables MC, Mann CH, Martin C, Philp A, Baar K, Jeukendrup AE**. Training with low muscle glycogen enhances fat metabolism in well-trained cyclists. *Med Sci Sports Exerc* 42(11): 2046-2055, 2010.

28. **Jackman SR, Witard OC, Jeukendrup AE, Tipton KD**. Branched-chain amino acid ingestion can ameliorate soreness from eccentric exercise. *Med Sci Sports Exerc* 42(5): 962-970, 2010.

29. **Jackson AS, Pollock ML**. Practical assessment of body composition. *Phys Sports Med* 13 :76-90, 1985.

30. **Jones DA**. High-and low-frequency fatigue revisited. *Acta Physiol Scand* 156: 265-270, 1996.

31. **Kanda K, Sugama K, Hayashida H, Sakuma J, Kawakami Y, Miura S, Yoshioka H, Mori Y, Suzuki K**. Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. *Exerc Immunol Rev* 19: 72-85, 2013.

32. **Keller C, Steensberg A, Hansen AK, Fischer CP, Plomgaard P, Pedersen BK**. Effect of exercise, training, and glycogen availability on IL-6 receptor expression in human skeletal muscle. *J Appl Physiol* 99: 2075-2079, 2005.

33. **Krishnan RK, Evans WJ, Kirwan JP**. Impaired substrate oxidation in healthy elderly men after eccentric exercise. *J Appl Physiol* 94: 716-723, 2003.

34. **Kuster M, Sakurai S, Wood GA**. Kinematic and kinetic comparison of downhill and level walking. *Clin Biomech* 10: 79-84, 1995.

35. **Langberg H, Olesen JL, Gemmer C, Kjaer M**. Substantial elevation of interleukin-6 concentration in peritendinous tissue, in contrast to muscle, following prolonged exercise in humans. *J Physiol* 542: 985-990, 2002.

36. **Langfort J, Zarzeczny R, Pilis W, Nazar K, Kaciuba-Uścitko H**. The effect of a low-carbohydrate diet on performance, hormonal and metabolic responses to a 30-s bout of supramaximal exercise. *Eur J Appl Physiol Occup Physiol* 76(2): 128-133, 1997.

37. **Leggate M, Nowell MA, Jones SA, Nimmo MA**. The response of interleukin-6 and soluble interleukin-6 receptor isoforms following intermittent high intensity and continuous moderate intensity cycling. *Cell Stress Chaperones* 15: 827-833, 2010.

38. **Lepers R, Hausswirth C, Maffiuletti, N, Brisswalter J, van Hoecke J**. Evidence of neuromuscular fatigue after prolonged cycling exercise. *Med Sci Sports Exerc* 32: 1880-1886, 2000.

39. **MacIntyre DL, Sorichter S, Mair J, Berg A, McKenzie DC**. Markers of inflammation and myofibrillar proteins following eccentric exercise in humans. *Eur J Appl Phys* 84: 180-186, 2001.

759 40. **Mackey AL, Bojsen-Moller J, Qvortrup K, Langberg H, Suetta C, Kalliokoski KK,**
760 **Kjaer M, Magnusson SP**. Evidence of skeletal muscle damage following electrically
761 stimulated isometric muscle contractions in humans. *J Appl Physiol* 105: 1620-1627, 2008.
762 41. **Martin V, Millet GY, Lattier G, Perrod, L**. Effects of recovery modes after knee
763 extensor muscles eccentric contractions. *Med Sci Sports Exerc* 36: 1907-1915, 2004.
764 42. **Martin V, Millet GY, Martin A, Deley G, Lattier G**. Assessment of low-frequency
765 fatigue with two methods of electrical stimulation. *J Appl Physiol 97*: 1923-1929, 2004.
766 43. **Martin PG, Weerakkody N, Gandevia SC, Taylor JL**. Group III and IV muscle
767 afferents differentially affect the motor cortex and motoneurones in humans. *J Physiol* 586:
768 1277-1289, 2008.
769 44. **Millet GY, Lepers R**. Alterations of neuromuscular function after prolonged running,
770 cycling and skiing exercises. *Sports Med* 34: 105-116, 2004.
771 45. **Millet GY, Martin V, Lattier G, Ballay Y**. Mechanisms contributing to knee extensor
772 strength loss after prolonged running exercise. *J Appl Physiol* 94: 193-198, 2003.
773 46. **Newell J, Higgins D, Madden N, Cruickshank J, Einbeck J, McMillan K, McDonald**
774 **R**. Software for calculating blood lactate endurance markers. *J Sports Sci* 25: 1403-1409,
775 2007.
776 47. **Nielsen JS, Madsen K, Jørgensen LV, Sahlin K**. Effects of lengthening contraction on
777 calcium kinetics and skeletal muscle contractility in humans. *Acta Physiol Scand* 184: 203-
778 214, 2005.
779 48. **Nieman DC, Henson DA, Smith LL, Utter AC, Vinci DM, Davis JM. Kaminsky DE,**
780 **Shute M**. Cytokine changes after a marathon race. *J Appl Physiol* 91: 109-114, 2001.
781 49. **Nybo L, Nielsen B, Pedersen BK, Moller K, Secher NH**. Interleukin-6 release from the
782 human brain during prolonged exercise. *J Physiol* 542: 991-995, 2002.

50. **O'Reilly KP, Warhol MJ, Fielding RA, Frontera WR, Meredith CN, Evans WJ**. Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. *J Appl Physiol* 63: 252-256, 1987.

51. **Osborne MA, Schneider DA**. Muscle glycogen reduction in man: relationship between surface EMG activity and oxygen uptake kinetics during heavy exercise. *Exp Physiol* 91: 179-189, 2006.

52. **Ostapiuk-Karolczuk J, Zembron-Lacny A, Naczk M, Gajewski M, Kasperska A, Dziewiecka H, Szyszka K**. Cytokines and cellular inflammatory sequence in non-athletes after prolonged exercise. *J Sports Med Phys Fitness*, 52: 563-568, 2012.

53. **Ostrowski K, Hermann C, Bangash A, Schjerling P, Nielsen JN, Pedersen, BK**. A trauma-like elevation of plasma cytokines in humans in response to treadmill running. *J Physiol* 513 (Pt 3): 889-894, 1998.

54. **Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK**. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J Physiol* 515: 287-291, 1999.

55. **Paulsen G, Mikkelsen UR, Raastad T, Peake JM**. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exerc Immunol Rev* 18: 42-97, 2012.

56. **Peake J, Nosaka K, Suzuki K**. Characterization of inflammatory responses to eccentric exercise in humans. *Exerc Immunol Rev* 11: 64-85, 2005.

57. **Pedersen BK, Febbraio MA**. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev* 88: 1379-1406, 2008.

58. **Pedersen BK**. Muscular interleukin-6 and its role as an energy sensor. *Med Sci Sports Exerc* 44(3): 392-396, 2012.

807 59. **Piehl K**. Time course for refilling of glycogen stores in human muscle fibres following
808 exercise-induced glycogen depletion. *Acta Physiol Scand* 90: 297-302, 1974.
809 60. **Pilegaard H, Keller C, Steensberg A, Helge JW, Pedersen BK, Saltin B. Neufer PD**.
810 Influence of pre-exercise muscle glycogen content on exercise-induced transcriptional
811 regulation of metabolic genes. *J Physiol* 541: 261-271, 2002.
812 61. **Place N, Maffiuletti NA, Martin A, Lepers R**. Assessment of the reliability of central
813 and peripheral fatigue after sustained maximal voluntary contraction of the quadriceps
814 muscle. *Muscle Nerve* 35: 486-495, 2007.
815 62. **Prather AA, Rabinovitz M, Pollock BG, Lotrich FE**. Cytokine-induced depression
816 during IFN-alpha treatment: the role of IL-6 and sleep quality. *Brain Behav Immun* 23: 1109-
817 1116, 2009.
818 63. **Morgan DL, Allen DG**. Early events in stretch-induced muscle damage. *J Appl Physiol*
819 *(1985)* 87(6): 2007-2015, 1999.
820 64. **Proske U, Morgan DL**. Muscle damage from eccentric exercise: mechanism,
821 mechanical signs, adaptation and clinical applications. *J Physiol* 537: 333-345, 2001.
822 65. **Psilander N, Frank P, Flockhart M, Sahlin K**. Exercise with low glycogen increases
823 PGC-1α gene expression in human skeletal muscle. *Eur J Appl Physiol* 113(4): 951-63, 2013.
824 66. **Racinais S, Bringard A, Puchaux K, Noakes TD, Perrey S**. Modulation in voluntary
825 neural drive in relation to muscle soreness. *Eur J Appl Physiol* 102: 439-446, 2008.
826 67. **Reihmane D, Jurka A, Tretjakovs P, Dela F**. Increase in IL-6, TNF-alpha, and MMP-
827 9, but not sICAM-1, concentrations depends on exercise duration. *Eur J Appl Physiol* 113:
828 851-858, 2013.
829 68. **Rijkelijkhuizen JM, de Ruiter CJ, Huijing PA, de Haan A**. Low-frequency fatigue,
830 post-tetanic potentiation and their interaction at different muscle lengths following eccentric
831 exercise. *J Exp Biol* 208: 55-63, 2005.

69. **Robson-Ansley PJ, de Milander L, Collins M, Noakes TD**. Acute interleukin-6 administration impairs athletic performance in healthy, trained male runners. *Can J Appl Physiol* 29: 411-418, 2004.

70. **Saxton JM, Clarkson PM, James R, Miles M, Westerfer M, Clark S, Donnelly AE**. Neuromuscular dysfunction following eccentric exercise. *Med Sci Sports Exerc* 27(8): 1185-1193, 1995.

71. **Scott JP, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD**. Effect of exercise intensity on the cytokine response to an acute bout of running. *Med Sci Sports Exerc* 43(12): 2297-306, 2011.

72. **Scott JP, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD**. Cytokine response to acute running in recreationally-active and endurance-trained men. *Eur J Appl Physiol* 113: 1871-1882, 2013.

73. **Shield A, Zhou S**. Assessing voluntary muscle activation with the twitch interpolation technique. *Sports Med* 34: 253-267, 2004.

74. **Siri WE**. The gross composition of the body. *Adv Biol Med Phys* 4: 239-280, 1956.

75. **Starkie RL, Arkinstall MJ, Koukoulas I, Hawley JA, Febbraio MA**. Carbohydrate ingestion attenuates the increase in plasma interleukin-6, but not skeletal muscle interleukin-6 mRNA, during exercise in humans. *J Physiol* 533: 585-591, 2001.

76. **Stouthard JM, Romijn JA, Van der Poll T, Endert E, Klein S, Bakker PJ, Veenhof CH, Sauerwein HPL**. Endocrinologic and metabolic effects of interleukin-6 in humans. *Am J Physiol* 268: E813-819, 1995.

77. **Strojnik V, Komi PV**. Neuromuscular fatigue after maximal stretch-shortening cycle exercise. *J Appl Physiol* 84: 344-350, 1998.

78. **Takekura H, Fujinami N, Nishizawa T, Ogasawara H, Kasuga N**. Eccentric exercise-induced morphological changes in the membrane systems involved in excitation-contraction coupling in rat skeletal muscle. *J Physiol* 533: 571-583, 2001.

79. **Thomas JR, Salazar W, Landers DM**. What is missing in p less than .05? Effect size. *Res Q Exerc Sport* 62: 344-348, 1991.

80. **Thomson JA, Green HJ, Houston ME**. Muscle glycogen depletion patterns in fast twitch fibre subgroups of man during submaximal and supramaximal exercise. *Pflugers Arch* 379: 105-108, 1979.

81. **Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B, Millet GY**. Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. *J Appl Physiol* 106: 701-710, 2009.

82. **Westerblad H, Duty S, Allen DG**. Intracellular calcium concentration during low-frequency fatigue in isolated single fibers of mouse skeletal muscle. *J Appl Physiol* 75: 382-388, 1993.

Figure Captions

Figure 1. Schematic of the experimental procedures. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). Arrows indicate the time points for recordings of neuromuscular function, muscle soreness and blood sampling for glucose, lactate and IL-6. 14 h-pre was baseline before the cycling protocol initiating the reduced glycogen condition or rest for the normal glycogen condition. 12 h-pre was after the cycling protocol or rest. 1 h-pre

was before the downhill running protocol. Participants underwent cycling or rest the evening before next morning's downhill running in random order and six weeks apart.

Figure 2. Isometric maximal voluntary contraction (iMVC) force of the knee extensors in normal and reduced glycogen conditions. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol initiating the reduced glycogen condition or rest for the normal glycogen condition. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD. * Significant difference between conditions, $ Significant difference from baseline (i.e. 14 h-pre) for the reduced glycogen condition, # Significant difference from baseline (i.e. 14 h-pre) for the normal condition, $P<0.05$.

Figure 3. Doublet peak force of the knee extensors in normal and reduced glycogen conditions. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol for the reduced glycogen condition or rest for the normal glycogen condition. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD. * Significant difference between conditions, $ Significant difference from baseline (i.e. 14 h-pre) for the reduced glycogen condition, # Significant difference from baseline (i.e. 14 h-pre) for the normal condition, $P<0.05$.

Figure 4. Doublet contraction time of the knee extensors in normal and reduced glycogen conditions. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol for the reduced glycogen condition or rest for

the normal glycogen condition. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD.

Figure 5. Doublet average rate of force development of the knee extensors in normal and reduced glycogen conditions. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol for the reduced glycogen condition or rest for the normal glycogen condition. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD.

Figure 6. Doublet half relaxation time of the knee extensors in normal and reduced glycogen conditions. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol for the reduced glycogen condition or rest for the normal glycogen condition. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD. $ Significant difference from baseline (i.e. 14 h-pre) for the reduced glycogen condition, # Significant difference from baseline (i.e. 14 h-pre) for the normal condition, $P<0.05$.

Figure 7. Voluntary activation (resting doublet) of the knee extensors in normal and reduced glycogen conditions. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol for the reduced glycogen condition or rest for the normal glycogen condition. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD. $ Significant difference from baseline (i.e. 14 h-pre) for the reduced glycogen condition, # Significant difference from baseline (i.e. 14 h-pre) for the normal condition, $P<0.05$.

Figure 8. Voluntary activation (potentiated doublet) of the knee extensors in normal and reduced glycogen conditions. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol for the reduced glycogen condition or rest for the normal glycogen condition. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD. $ Significant difference from baseline (i.e. 14 h-pre) for the reduced glycogen condition, # Significant difference from baseline (i.e. 14 h-pre) for the normal condition, $P<0.05$.

Figure 9. 20 Hz force response for normal and reduced glycogen conditions following eccentric exercise. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol or rest. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD. * Significant difference between conditions, $ Significant difference from baseline (i.e. 14 h-pre) for the reduced glycogen condition, # Significant difference from baseline (i.e. 14 h-pre) for the normal condition., $P<0.05$.

Figure 10. 50 Hz force response for normal and reduced glycogen conditions following eccentric exercise. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol or rest. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD.

Figure 11. Low-to-high frequency ratio change in normal and reduced glycogen conditions following eccentric exercise. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol or rest. 12 h-pre was after

the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD. $ Significant difference from baseline (i.e. 14 h-pre) for the reduced glycogen condition, # Significant difference from baseline (i.e. 14 h-pre) for the normal condition, * Significant difference between conditions, $P<0.05$.

Figure 12. Perceived muscle soreness in rest of the knee extensors in normal and reduced glycogen conditions. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol for the reduced glycogen condition or rest for the normal glycogen condition. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD. * Significant difference between conditions, $ Significant difference from baseline (i.e. 14 h-pre) for the reduced glycogen condition, # Significant difference from baseline (i.e. 14 h-pre) for the normal condition, $P<0.05$.

Figure 13. Perceived muscle soreness during contraction of the knee extensors in normal and reduced glycogen conditions. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol for the reduced glycogen condition or rest for the normal glycogen condition. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD. * Significant difference between conditions, $ Significant difference from baseline (i.e. 14 h-pre) for the reduced glycogen condition, # Significant difference from baseline (i.e. 14 h-pre) for the normal condition, $P<0.05$.

Figure 14. Perceived muscle soreness during stretch of the knee extensors in normal and reduced glycogen conditions. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol for the reduced glycogen condition or rest for the normal glycogen condition. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD. * Significant difference between conditions, $ Significant difference from baseline (i.e. 14 h-pre) for the reduced glycogen condition, # Significant difference from baseline (i.e. 14 h-pre) for the normal condition, $P<0.05$.

Figure 15. Plasma interleukin-6 (IL-6) concentration measured under normal and reduced glycogen conditions following eccentric exercise. The end of the downhill run was selected as the zero time point (i.e. 0 h-post). 14 h-pre was baseline before the cycling protocol or rest. 12 h-pre was after the cycling protocol or rest. 1 h-pre was before the downhill running protocol. Data are presented as mean ± SD. * Significant difference between conditions, $ Significant difference from baseline (i.e. 14 h-pre) for the reduced glycogen condition, # Significant difference from baseline (i.e. 14 h-pre) for the normal condition, $P < 0.05$.