Cardiorespiratory and metabolic responses after exercise-induced muscle damage: the influence of lowered glycogen

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ABSTRACT

BACKGROUND: We examined the effect of early-onset of muscle damage and low muscle glycogen on cardiorespiratory and metabolic responses to low-intensity exercise.

METHODS: Twelve men cycled for 10 min at 50% maximal oxygen uptake before, and 12 h after a morning downhill run (five, 8 min bouts at -12% gradient, with 2 min rests) under normal (NORM) and lowered glycogen (LOW) conditions, following a crossover design with conditions separated by six weeks. Cardiorespiratory responses were recorded, with oxidation measures derived from stoichiometry equations.

RESULTS: Muscle damage symptoms post-downhill (0 h) were similar between conditions. Carbon dioxide ventilatory equivalent increased 12 h post-downhill for LOW ($P<0.05$), but not NORM ($P=0.7$). A trend towards decreased respiratory exchange ratio (RER) was shown 12 h post-downhill for LOW (1.00±0.07 to 0.89±0.12, $P=0.06$), but not NORM (0.94±0.11 to 0.94±0.08; $P=0.6$). Twelve hours after LOW downhill running fat oxidation increased (0.21±0.18 $g\cdot min^{-1}$ to 0.36±0.27 $g\cdot min^{-1}$; $P<0.05$) and carbohydrate oxidation decreased (2.68±0.52 $g\cdot min^{-1}$ to 1.98±0.75 $g\cdot min^{-1}$; $P<0.05$); NORM oxidation rates were unchanged (fat: 0.26±0.18 $g\cdot min^{-1}$ to 0.33±0.18 $g\cdot min^{-1}$; $P=0.5$; carbohydrate: 2.51±0.49 $g\cdot min^{-1}$ to 2.29±0.47 $g\cdot min^{-1}$; $P=0.3$).

CONCLUSION: Cycling at low-intensity 12 h post-downhill running with lowered muscle glycogen increased fat oxidation, decreased carbohydrate oxidation and elevated carbon dioxide ventilation. Damaging exercise with reduced glycogen availability increases fat utilization during subsequent low-intensity exercise as little as 12 h later.

Key words: Downhill running; glycogen availability; fat oxidation; low-intensity exercise; muscle soreness.

Introduction

Repeated, intense and/or prolonged eccentric contractions are common in daily life, including stair descent, sitting down, and running. These actions can result in exercise-induced muscle damage, with acute force loss, muscle soreness and disrupted glucose metabolism.[1, 2] Cardiorespiratory and metabolic responses during subsequent exercise may also be altered, and at higher intensities, athletic performance impaired.[3-5] The susceptibility to damage[6] and greater glycogen utilization rate[2] of type II fibers in response to eccentric exercise, may contribute to compromised exercise performance. Metabolism during exercise when muscle is damaged, may also be altered by inflammation,[7] reduced glucose uptake[1] and reduced glycogen resynthesis.[3]

Endurance exercise capacity is impaired up to 48 h after muscle damage,[4, 8] with increases in oxygen cost,[9] blood lactate, respiratory exchange ratio (RER)[10] and minute ventilation ($\dot{V}_E$).[8] Elevations in ventilation and effort perception when cycling 48 h after eccentric exercise appear intensity-dependent,[11] and are attributed to increased circulating lactate, and by implication, greater type II fiber recruitment.[12]

The increased physiological stress when exercising over repeat days with muscle damage, may compromise subsequent performance.[4] However, whether exercise-induced ventilatory and metabolic responses are altered at lower exercise intensities is not known. This has relevance to those alternating between resistance and aerobic exercises, undertaking high-volume training and bouts within-, and between-days.

Hughes et al.[5] associated eccentric exercise-induced strength loss and increased muscle soreness with greater carbohydrate oxidation, as opposed to fat metabolism, during subsequent concentric exercise. However, at rest, others have reported elevated fat oxidation and energy expenditure in young women,[13] and decreased fat oxidation and preserved carbohydrate oxidation in young men[14] following eccentric knee extensions. Higher fat oxidation in women, than in men, during exercise of different intensities and modes[15] may be partly attributable to a greater proportion of oxidative, type I fibers in women.[16] However, potential gender-dependent effects on fat oxidation during eccentric exercise remain vague. If downhill running-induced muscle damage leads to preferential type II fiber damage, thus delaying glycogen repletion,[2] then carbohydrate oxidation may decrease, and fat oxidation increase, for subsequent activity. Downhill running with lowered muscle glycogen may disrupt substrate metabolism further (by reduced carbohydrate availability and elevated fat utilization), in turn, augmenting the cardiorespiratory response to exercise. We hypothesize that commencing downhill running with lowered glycogen would augment alterations in fat and carbohydrate oxidation, resulting in reliance upon fat, at the expense of carbohydrate oxidation. The study purpose was to investigate the effect of muscle damaging exercise with lowered glycogen, on cardiorespiratory and metabolic responses during low-intensity concentric exercise performed 12 h later.

Materials and methods

Participants

Twelve non-smoking, healthy males (mean ± SD: age, 23 ± 4 years; height, 179 ± 5 cm; body mass, 77 ± 10 kg; body fat, 14.4 ± 3.8%) volunteered for participation in the study. All were physically and recreationally active (maximal oxygen uptake ($\dot{V}O_{2max}$), 54.3 ± 9.1 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$), and had no history of structured resistance and/or regular running training. Participants were normal weight (body mass index, <25 $kg{\cdot}m^{-2}$) according to the World Health Organisation, free from cardiorespiratory disorders, and were not using anti-inflammatory medicines during the experimental period. The University of Chichester Research Ethics Committee granted approval for the study, and the experimental procedures conformed to the Helsinki Declaration. All procedures, and the associated risks and benefits, were fully explained to participants before written informed consent was obtained for their participation.

Experimental design

Participants completed two pre-experimental, familiarization sessions 48 h apart, and at least 7 days before completing a three session experimental protocol. Familiarizations occurred before the first condition only: either normal muscle glycogen (NORM), or lowered muscle glycogen (LOW), which were separated by at least 6 weeks in a randomized cross-over design. The three experimental sessions were performed over two consecutive days (Figure 1): Session 1 (day 1): 10 min low-intensity cycling followed by glycogen manipulation (LOW) or quiet rest (NORM); Session 2 (day 2): a downhill run; Session 3 (day 2): 12 h post-downhill 10 min low-intensity cycling measurement. The LOW involved an exhaustive, cycling exercise evening session, followed by a morning downhill run (fasted from 3 h pre-cycling to 1 h post-downhill run); NORM involved a resting evening session, followed by a morning downhill run. Oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and heart rate (HR) were recorded during all low-intensity cycling sessions.

Dietary control

Instruction was given to consume a low carbohydrate diet (total calorie intake ~3620 kJ: ~1% carbohydrate, ~24% protein, ~75% fat) between the LOW downhill run and the 12 h post-downhill measurement. Habitual diet was maintained and self-recorded from 48 h prior to the first experimental condition, up to 48 h after the downhill run (total calorie intake per day ~8586 kJ: ~51% carbohydrate, ~25% protein, ~24% fat). Food records were analyzed with nutritional software (Nutritics Ltd, Co. Dublin, Ireland), checked upon each visit, and prescribed for the subsequent condition; physical activity was also requested to remain low between conditions.[17]

<<< INSERT FIGURE 1. HERE >>>

Pre-experimental familiarization sessions

Two, pre-experimental familiarization sessions commenced with anthropometric measurements. Height and mass were measured unshod, and then skinfold thickness was quantified with a Harpenden calliper (Baty Int., West Sussex, UK) to estimate body density [18] and body composition.[19]

Familiarization one involved an incremental cycling trial, with participants cycling (Excalibur Sport 925900, Lode, Groningen, The Netherlands) at ~75 rpm for 3 min at 50 W; thereafter, power was increased by 10 W every 20 s, until volitional exhaustion. Breath-by-breath $\dot{V}O_2$ and $\dot{V}CO_2$ were sampled using a portable metabolic cart, calibrated following manufacturer's instructions (Cosmed K4b^2, Rome, Italy), and HR (Polar Electro Oy, Kempele, Finland) was measured continuously. The highest 15 s average for $\dot{V}O_2$ was taken as $\dot{V}O_{2max}$ and the corresponding power recorded ($\dot{V}O_{2max}$ power 324 ± 57 W) and used to establish experimental cycling workloads.

Familiarization two involved a submaximal, incremental treadmill run (Pulsar, h/p/cosmos Sports & Medical GmbH, Germany) to establish individual downhill running speed (based upon lactate threshold).[20] The run began at 8 km·h^{-1} (1% gradient), followed by 1 km·h^{-1} increments every 4 min until volitional exhaustion (the point at which the participant felt they could no longer continue), or eight stages were completed. Fingertip blood (25µL) samples were drawn from the right index finger, with the pronated hand resting on the treadmill handrail. This ensured sufficient blood for duplicate lactate analysis for each stage (2300 STAT Plus™ analyzer, YSI Life Sciences, Yellow Springs, USA); subsequent values were used to determine running speed at lactate threshold for individual participants.

Experimental sessions

Session 1 (day 1) began for both conditions with participants attending the laboratory after 19:00 hrs, in a 3 h fasted state and completed 10 min of constant-load cycling. Breath-by-breath $\dot{V}O_2$ and $\dot{V}CO_2$, and HR were measured. Cadence was maintained at ~75 rpm, with the bout preceded by 1 min cycling at 50 W, before increasing to the required ~50% $\dot{V}O_{2max}$ power (163 ± 38 W). The cycling duration was limited to avoid influencing the cardiorespiratory responses to downhill running and responses up to 2 min excluded as participants were unlikely to have achieved a steady-state.

For the LOW condition, participants then cycled at 60% $\dot{V}O_{2max}$ power (~75 rpm; workload, 181 ± 40 W) until volitional exhaustion (time, 95 ± 13 min; blood glucose reduced by -1.47 ± 0.56 $mmol{\cdot}L^{-1}$ (-31.8%)).[20] Biopsy studies have shown this protocol to be effective for depleting muscle glycogen (reduction: total muscle, -77%, type I fibers, -95%, type II fibers, -70%).[21, 22] For the NORM condition, participants completed a 2 h seated quiet rest, with no change in blood glucose values.

Session 2 (day 2) began for both conditions (~07:00 hrs) with five, 8 min downhill runs at lactate threshold speed (-12% gradient, 12.1 ± 1.1 $km{\cdot}h^{-1}$) each separated by 2 min level jogging (1% gradient at 8 $km{\cdot}h^{-1}$).[23] The LOW run was performed with decreased blood glucose compared to pre-exhaustive cycling (pre-run, -23.2%; post-run, -26.0%, both $P < 0.01$); the NORM run was performed with normal blood glucose compared to pre-quiet rest values (pre-run, -2.7%; post-run, 7.3%).[20] Muscle force and soreness measurements were repeated immediately after completion of the run.

Session 3 (day 2) was conducted 12 h post-downhill run with the 10 min cycling bout, and then muscle force and soreness measurements repeated in order.

Calculation of substrate oxidation

Breath-by-breath data were averaged over 15 s periods for: tidal volume, $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}O_2$ (mL·kg^{-1}·min^{-1}), RER, HR and ventilatory equivalents of oxygen ($\dot{V}_E/\dot{V}O_2$), and carbon dioxide ($\dot{V}_E/\dot{V}CO_2$). Substrate oxidation calculations were taken for moderate intensity exercise, assuming negligible urinary nitrogen rate,[24] and made using Equations 1 and 2:

1) Fat oxidation (g·min^{-1}) = 1.695 x $\dot{V}O_2$ – 1.701 x $\dot{V}CO_2$

2) Carbohydrate oxidation (g·min^{-1}) = 4.344 x $\dot{V}CO_2$ – 3.061 x $\dot{V}O_2$

Cardiorespiratory responses refer to: HR, tidal volume, $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$; metabolic responses refer to: RER, fat oxidation and carbohydrate oxidation.

Muscle force loss and soreness measurement

Isometric maximal voluntary contraction (MVC) and muscle soreness of the knee extensors were used to indirectly indicate muscle damage.[25] Maximal force and soreness were assessed on a custom-built strength-testing chair (University of Chichester, UK) in familiarization one and, immediately (0 h) and 12 h after each downhill run. Seated and secured with the hip and knee at 90°, participants had a steel chain attached proximally to the fibular notch and medial malleolus with padding, leading to a mechanically calibrated S-beam load-cell (RS 250 kg, Tedea Huntleigh, Cardiff, UK) beneath the chair. A personal computer displayed instantaneous force output at 1000 Hz (Chart 4 v 4.1.2, AD Instruments, Oxford, UK) during maximal contraction. Soreness was determined before MVC using a visual analogue scale (0, not at all sore; 10, extremely sore), whilst undergoing muscle-belly palpation (until the investigator exerted enough pressure to blanch the fingernail).[26] Isometric MVC was measured using three separate, 3 to 5 s contractions, with 2 min rests. Knee extensor force loss after downhill running did not show an order effect at 0 h ($P = 0.2$) or 12 h later ($P = 0.3$). The investigator provided verbal encouragement; a chair-linked computer monitor provided force-time feedback.

Statistical analysis

A two-way, repeated measures analysis of variance (ANOVA; condition and time) was used for each cardiorespiratory, metabolic and muscle-damage measure between conditions. For low-intensity cycling, cardiorespiratory measures are presented for the final minute (9 to 10 min), and metabolic measures every 2 min, from minute two onwards (i.e., 3 to 4 min, 5 to 6 min, 7 to 8 min and 9 to 10 min), due to time to reach steady-state. Pre-planned, paired t-tests, with a Bonferroni correction were used to locate specific differences. A Greenhouse-Geisser correction was applied where assumptions of sphericity were violated. Analyses were calculated with IBM SPSS Statistics, version 20 (IBM Corp, Armonk, NY), with data presented as mean ± SD and statistical significance set at $P < 0.05$. A statistical trend was interpreted as $0.05 > P < 0.1$ according to Curran-Everett and Benos.[27]

Results

Muscle force loss and soreness

Baseline knee extensor force and muscle soreness were similar between conditions ($P > 0.05$; Table I). Muscle damage was evidenced after downhill running by an immediate force loss ($P < 0.0001$) of -27.3% in the NORM (-178.5 N) and -29.5% in the LOW (-195.5 N); and a 12 h post force loss ($P < 0.001$) of -15.5% in the NORM (-101.5 N) and -15.3% in the LOW (-101.6 N). Muscle soreness increased ($P < 0.01$) similarly between conditions immediately (NORM, 3.8 ± 1.9; LOW, 2.8 ± 1.4), and 12 h after downhill running (Table I).

<<< INSERT TABLE I. HERE >>>

Cardiorespiratory measures

After downhill running there was no change in tidal volume ($F_{(1,11)} = 0.6$, $P = 0.5$), $\dot{V}_E$ ($F_{(1,11)} = 0.3$, $P = 0.6$), $\dot{V}O_2$ ($F_{(1,10)} = 0.05$, $P = 0.8$), $\dot{V}CO_2$ ($F_{(1,9)} = 0.66$, $P = 0.4$), $\dot{V}O_2$ (mL·kg^{-1}·min^{-}

^{1}) ($F_{(1,11)} = 0.1$, $P = 0.9$) or HR ($F_{(1,11)} = 0.2$, $P = 0.7$) during low-intensity cycling in both conditions (Table I). $\dot{V}_E/\dot{V}CO_2$ was elevated by 3.4 L·min^{-1} for LOW ($F_{(1,11)} = 2.6$, $P < 0.05$), but unchanged for NORM ($P = 0.7$; Table I). A moderate condition-time effect was shown for $\dot{V}_E/\dot{V}O_2$ ($F_{(1,11)} = 5.9$, $P < 0.05$) and LOW, although $\dot{V}_E/\dot{V}O_2$ did not change for NORM ($P = 0.4$) or LOW ($P = 0.1$).

Metabolic measures

Baseline RER was similar between conditions when commencing cycling (at 4 min: NORM, 0.97 ± 0.11; LOW, 1.03 ± 0.07, $P = 0.09$). A condition-time effect ($F_{(1,11)} = 5.9$, $P < 0.05$) was shown for RER in LOW; with no change for NORM (pre 0.94 ± 0.11, 12 h post-downhill 0.94 ± 0.08, $P = 0.6$; Figure 2A) and a trend towards lower RER for LOW (pre 1.00 ± 0.07, 12 h post-downhill 0.89 ± 0.12, $P = 0.06$; Figure 2B). Fat oxidation was unchanged for NORM ($P = 0.5$; Figure 3A), but increased ($P < 0.05$) by 0.15 g·min^{-1} for LOW (Figure 3B). Carbohydrate oxidation was unchanged for NORM ($P = 0.3$; Figure 4A), but decreased ($P < 0.05$) by 0.72 g·min^{-1} for LOW (Figure 4B).

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Discussion

Our main purpose was to examine the effect of downhill running, with lowered muscle glycogen, on cardiorespiratory and metabolic responses during subsequent concentric exercise. Twelve hours after downhill running, muscle damage was confirmed in both conditions by force loss and muscle soreness. A bout of downhill running had little effect on cardiorespiratory response, but did alter ventilatory equivalents and substrate metabolism when cycling at low-intensity 12 h later. Increased fat oxidation and decreased carbohydrate oxidation when exercising in a lowered glycogen, muscle damaged state, is supported by a trend for lower RER. Conversely, RER was similar during cycling exercise with normal glycogen and muscle damage.

Previous studies investigating the influence of muscle damage on aerobic, metabolic and endurance performance have focussed upon the 24 to 48 h post-exercise period.[10, 11, 28] Our study is novel as we examined 12 h post-downhill running to determine whether emerging muscle damage can alter cardiorespiratory and metabolic function at low exercise intensities. This is important, as low-intensity activity is often prescribed to alleviate symptoms of muscle damage during heavy training periods.[29]

Cardiorespiratory responses were mostly unaffected 12 h after downhill running regardless of glycogen state. However, $\dot{V}_E/\dot{V}CO_2$ increased with large effect after LOW downhill running, suggesting incomplete glycogen recovery 12 h later. A 3.4 $L \cdot min^{-1}$ rise at the same cycling workload for LOW indicates increased ventilation, relative to carbon dioxide production, suggesting increased bicarbonate buffering of accumulating hydrogen ions (H^+).[30] It should be noted that $\dot{V}_E/\dot{V}CO_2$ increased for LOW i) sub-ventilatory threshold, ii) at a single time-point prior to peak-damage (i.e., 12 h post-downhill run) and iii) in the final minute of short duration low-intensity cycling. Typically, exercise-induced force losses by muscle damage are accompanied by leakage of intracellular enzymes,[1] including lactate dehydrogenase (LDH) and creatine kinase. The loss of LDH may disrupt substrate metabolism (increase H^+ accumulation and buffering), in turn, elevating ventilation, relative to $\dot{V}CO_2$. Had lowered glycogen greater effect, ventilatory alterations would have manifested from the onset of low-intensity cycling and at a greater magnitude. Furthermore, RER became significantly lower for LOW only in the final minutes, suggesting that metabolic steady-state requires at least 8 min during low-intensity exercise. Increased $\dot{V}_E/\dot{V}CO_2$, with decreased $\dot{V}_E/\dot{V}O_2$, has been shown for glycogen depleted young men, during incremental cycling.[31] As exercise intensity increases, elevations in $\dot{V}_E/\dot{V}CO_2$ and blood lactate coincide. However, $\dot{V}_E/\dot{V}O_2$ would typically increase first. With further increases in intensity, ventilation rises disproportionately with carbon dioxide production to elevate $\dot{V}_E/\dot{V}CO_2$. Like Hughes et al.,[31] we found lowering muscle glycogen led to $\dot{V}_E/\dot{V}CO_2$ rising at a lower cycling workload, when compared to NORM. However, our exercise intensity was constant-load and below the ventilatory threshold, as indicated by power output and ventilatory equivalents. We suspect that increased $\dot{V}_E/\dot{V}CO_2$ when cycling with LOW, 12 h after muscle damage, may be due to: greater type II fiber recruitment, LDH impairment and slightly elevated blood lactate concentrations. Stable $\dot{V}_E/\dot{V}O_2$ with increased $\dot{V}_E/\dot{V}CO_2$ suggests ventilation can satisfy muscle oxygen delivery, but not carbon dioxide removal. Assuming glycogen-lowering cycling depleted type I fibers, our LOW condition would involve greater type II fiber recruitment. Additional type II recruitment (coupled with enzyme leakage) with LOW could have increased H^+ accumulation and buffering (H^+ and HCO_{3^-} convert to CO_2 and H_2O at the lungs), and subsequently expired CO_2.

Unexpectedly $\dot{V}_E/\dot{V}O_2$ was not influenced by muscle damage or with lowered glycogen. It was unlikely that gas sampling data 'smoothing' may have hidden minor $\dot{V}_E/\dot{V}O_2$ fluctuations, as $\dot{V}_E/\dot{V}CO_2$ did change. Paschalis et al.[32] found muscle damage did not influence $\dot{V}_E$, $\dot{V}O_2$, HR and RER during running (at 55 and 75% $\dot{V}O_{2max}$) up to 96 h later. Conversely, cycling for 20 min at 50% $\dot{V}O_{2max}$ (139 ± 4 W) in a glycogen-depleted state (by 3 h cycling at ~40% $\dot{V}O_{2max}$) has been shown to increase $\dot{V}O_2$ and HR, and reduce RER in young adults.[33] Cardiorespiratory function was unaltered in our study, potentially due to steady-state (at low relative workload) muscle O_2 consumption causing little change in pulmonary gas exchange. The 10 min measurement period may have been too brief to measure progression from the first phase of ventilatory dynamics.[34] Low-intensity cycling also commenced from a 'baseline' 50 W workload, which in comparison to cycling from rest, may induce a less abrupt rise in cardiorespiratory response.[34] Elsewhere, Vassilis et al.[35] showed evidence of muscle damage, without altered cardiorespiratory responses when running at 70% $\dot{V}O_{2max}$. Conversely, muscle damage induced by squatting exercise has resulted in greater $\dot{V}_E$, but unchanged perceived exertion, for moderate-intensity cycling (80% ventilatory threshold) 48 h later.[11] For heavy-intensity cycling both $\dot{V}_E$ and perceived exertion increased. Squatting exercise has also been shown to increase $\dot{V}O_2$, $\dot{V}_E$ and HR for 10 min of lactate turn-point running, 24 and 48 h later.[28] The influence of muscle damage on exercise performance appears not only dependent upon the eccentric bout, but also the intensity of the subsequent activity.

A trend towards decreased RER was shown during LOW cycling 12 h after downhill running (0.89 ± 0.12, P = 0.06), when compared to baseline (1.00 ± 0.07). Subsequent exercise of higher-intensity would be required to demonstrate this shift from carbohydrate metabolism (requiring more CO_2, than O_2), to fat metabolism (requiring more O_2, than CO_2), when glycogen lowered. Fat oxidation involves more O_2 and CO_2, supporting an increased $\dot{V}_E/\dot{V}CO_2$ 12 h after LOW downhill running. Brief exercise duration meant that RER reduced significantly for LOW only in the final minutes. Limiting carbohydrate availability by dietary/exercise-manipulation (LOW) would increase the likelihood of accelerating free fatty-acid mobilization.[36] A subsequent pH decrease (acidosis)[37] would be expected to stimulate $\dot{V}_E/\dot{V}CO_2$.

Fat oxidation is the main metabolic contributor to low-intensity exercise[38] and is further stimulated with low muscle glycogen.[36, 39] Cheneviére et al.[40] found prior heavy exercise (90 min of constant-load 50% $\dot{V}O_{2max}$ cycling) increased fat oxidation during a subsequent submaximal, incremental test, more than light exercise (2.5 h seated rest). Their fat oxidation rates during submaximal exercise, pre- (0.30 g·min^{-1}) and post-light exercise (0.53 g·min^{-1}) were comparable to ours (NORM: pre 0.26 ± 0.18 g·min^{-1}, post-downhill 0.33 ± 0.18 g·min^{-}

[1]). However, we cannot discount the possibility that the evening, exhaustive exercise may have increased fat oxidation 12 h after the downhill run. Participants would have undergone the 12 h low-intensity cycling, approximately 22 h after the heavy-intensity cycling bout; still within the period of potential recovery (see Figure 1). Low-intensity exercise is often prescribed to remedy symptoms of muscle damage during intense training periods.[29] Therefore, understanding the cardiorespiratory and metabolic changes in the early onset of muscle damage has relevance to athletes and coaches, particularly when performing and recovering from consecutive exercise sessions. As muscle damage disturbs glucose uptake[1] and glycogen resynthesis,[3] lower rates of carbohydrate oxidation would be expected during subsequent exercise, preserving already reduced intramuscular substrates. Future research should look to examine substrate metabolism 12 to 48 h after whole-body eccentric exercise, from low to high intensity exercise. Main limitations to this study were that i) cardiorespiratory and metabolic measurements were constrained to low-intensity exercise at 12 h following eccentric-biased exercise, and ii) a pre-validated glycogen depletion protocol was performed,[22] but we could not directly assess glycogen reduction by biopsy.

What was surprising, given the increased fat oxidation, was the unchanged $\dot{V}O_2$ across conditions and time points. Fat metabolism has been found to incur greater oxygen demand, than carbohydrate oxidation, 4½ h after cycling (at ~57 $\dot{V}O_{2max}$);[41] therefore fat oxidation would be expected to rise with $\dot{V}O_2$. Estimation of substrate oxidation using indirect calorimetry is based upon $\dot{V}O_2$ and $\dot{V}CO_2$ measurement, which reflects whole-body metabolism. At best, accuracy is within 5% of muscle oxidation values at rest, and dietary status will influence substrate oxidation, regardless of the indirect calorimetry measurement sensitivity.[42]

We used an exhaustive cycling protocol, shown to reduce type I fiber glycogen by 95% in young men.[22] This may have contributed to greater preferential type II fiber recruitment and fiber damage during LOW downhill running, in comparison to NORM. Type II fiber recruitment is known to elevate $\dot{V}O_2$ more than type I, for cycling exercise.[33] Our $\dot{V}O_2$ remained similar to baseline, which may be explained by measurement during low-intensity exercise and/or preferential type II fiber damage.

Whole-body, eccentric exercise may not change cardiorespiratory and metabolic function for low-intensity activity in the emergence of muscle damage, but if damaging exercise is performed with lowered glycogen availability, substrate metabolism appears to shift to fat oxidation. These findings supplement our knowledge of the metabolic responses, and sensitivity to, eccentric-biased exercise.

Conclusions and implications

Our findings indicate that undergoing a bout of muscle-damaging exercise, with lowered muscle glycogen, can increase fat utilization and elevate carbon dioxide ventilation, even at low exercise intensities as little as 12 h after damaging exercise. An understanding of the metabolic and cardiorespiratory changes after damaging exercise, and their uncoupling across exercise intensities, has importance to those undertaking new, unaccustomed training regimen, as well as athletes/patients interspersing aerobic recovery exercise, into resistance training. These bouts may range from pre-season plyometric/sprint training, to recreational endurance events to untrained individuals skiing for leisure during vacation.

References

1. Costill DL, Pascoe DD, Fink WJ, Robergs RA, Barr SI, Pearson D. Impaired muscle glycogen resynthesis after eccentric exercise. J Appl Physiol 1990;69(1):46-50.
2. 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 1987;63(1):252-6.
3. Asp S, Daugaard JR, Kristiansen S, Kiens B, Richter EA. Exercise metabolism in human skeletal muscle exposed to prior eccentric exercise. J Physiol 1998;509(1):305-13.
4. Burt DG, Twist C. The effects of exercise-induced muscle damage on cycling time-trial performance. J Strength Condit Res 2011;25(8):2185-92.
5. Hughes J, Chapman P, Brown S, Johnson N, Stannard S. Indirect measures of substrate utilisation following exercise-induced muscle damage. Eur J Sport Sci 2013;13(5):1-9.
6. Friden J, Sjostrom M, Ekblom B. Myofibrillar damage following intense eccentric exercise in man. Int J Sports Med 1983;4(3):170-6.
7. Depner CM, Kirwan RD, Frederickson SJ, Miles MP. Enhanced inflammation with high carbohydrate intake during recovery from eccentric exercise. Eur J Appl Physiol 2010;109(6):1067-76.
8. Twist C, Eston RG. The effect of exercise-induced muscle damage on perceived exertion and cycling endurance performance. Eur J Appl Physiol 2009;105(4):559-67.
9. Braun WA, Dutto DJ. The effects of a single bout of downhill running and ensuing delayed onset of muscle soreness on running economy performed 48 h later. Eur J Appl Physiol 2003;90:29-34.
10. Gleeson M, Blannin AK, Zhu B, Brooks S, Cave R. Cardiorespiratory, hormonal and haematological responses to submaximal cycling performed 2 days after eccentric or concentric exercise bouts. J Sport Sci 1995;13(6):471-9.

11. Davies RC, Rowlands AV, Eston RG. Effect of exercise-induced muscle damage on ventilatory and perceived exertion responses to moderate and severe intensity cycle exercise. Eur J Appl Physiol 2009;107(1):11-9.
12. Chen TC, Nosaka K, Tu JH. Changes in running economy following downhill running. J Sport Sci 2007;25(1):55-63.
13. Paschalis V, Koutedakis Y, Baltzopoulos V, Mougios V, Jamurtas AZ. The effects of muscle damage on running economy in healthy males. Int J Sports Med 2005;26:827-31.
14. Krishnan RK, Evans WJ, Kirwan JP. Impaired substrate oxidation in healthy elderly men after eccentric exercise. J Appl Physiol 2003;94(2):716-23.
15. Knechtle B, Müller G, Willmann F, Kotteck K, Eser P, Knecht H. Fat oxidation in men and women endurance athletes in running and cycling. Int J Sports Med 2004;25(1):38-44.
16. Glenmark B. Skeletal muscle fibre types, physical performance, physical activity and attitude to physical activity in women and men. A follow-up from age 16 to 27. Acta Physiol Scand Suppl 1994;623:1-47.
17. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sport Exer 2003;35(8):1381-95.
18. Jackson AS, Pollock ML. Practical assessment of body composition. Phys Sportsmed 1985;13:76-90.
19. Siri WE. The gross composition of the body. Adv Biol Med Phys 1956;4:239-80.
20. Gavin JP, Myers SD, Willems MET. The effect of glycogen reduction on cardiorespiratory and metabolic responses during downhill running. Eur J Appl Physiol 2015;115(9):1125-33.

21. Osborne MA, Schneider DA. Muscle glycogen reduction in man: relationship between surface EMG activity and oxygen uptake kinetics during heavy exercise. Exp Physiol 2006;91(1):179-89.
22. Thomson JA, Green HJ, Houston ME. Muscle glycogen depletion patterns in fast twitch fibre subgroups of man during submaximal and supramaximal exercise. Pflugers Arch 1979;379(1):105-8.
23. 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 2000;10(4):199-204.
24. Jeukendrup AE, Wallis GA. Measurement of substrate oxidation during exercise by means of gas exchange measurements. Int J Sports Med 2005;26:S28-37.
25. Byrne C, Twist C, Eston, R. Neuromuscular function after exercise-induced muscle damage: theoretical and applied implications. Sports Med 2004;34(1):49-69.
26. Hickner RC, Mehta PM, Dyck D, Devita P, Houmard JA, Koves T et al. Relationship between fat-to-fat-free mass ratio and decrements in leg strength after downhill running. J Appl Physiol 2001;90(4):1334-41.
27. Curran-Everett D, Benos DJ. Guidelines for reporting statistics in journals published by the American Physiological Society. J Physiol 2004;97:457-9.
28. Burt D, Lamb K, Nicholas C, Twist C. Effects of repeated bouts of squatting exercise on sub-maximal endurance running performance. Eur J Appl Physiol 2013;113(2):285-93.
29. Suzuki M, Umeda T, Nakaji S, Shimoyama T, Mashiko T, Sugawara K. Effect of incorporating low intensity exercise into the recovery period after a rugby match. Br J Sports Med 2004;38(4):436-40.

30. Stringer W, Wasserman K, Casaburi R. The VCO2/VO2 relationship during heavy, constant work rate exercise reflects the rate of lactic acid accumulation. Eur J Appl Physiol Occup Physiol 1995;72(1-2):25-31.
31. Hughes EF, Turner SC, Brooks GA. Effects of glycogen depletion and pedaling speed on "anaerobic threshold". J Appl Physiol Respir Environ Exerc Physiol 1982;52(6):1598-1607.
32. Paschalis V, Nikolaidis MG, Theodorou AA, Panayiotou G, Fatouros IG, Koutedakis Y et al. A weekly bout of eccentric exercise is sufficient to induce health-promoting effects. Med Sci Sports Exerc 2011;43(1):64-73.
33. Krustrup P, Soderlund K, Mohr M, Bangsbo J. Slow-twitch fiber glycogen depletion elevates moderate-exercise fast-twitch fiber activity and O2 uptake. Med Sci Sports Exerc 2004;36(6):973-82.
34. Whipp BJ, Ward SA, Lamarra N, Davis JA, Wasserman K. Parameters of ventilatory and gas exchange dynamics during exercise. J Appl Physiol 1982;52(6):1506-13.
35. Vassilis P, Vassilios B, Vassilis M, Athanasios JZ, Vassilis T, Christina K et al. Isokinetic eccentric exercise of quadriceps femoris does not affect running economy. J Strength Condit Res 2008;22(4):1222-7.
36. Brooks GA, Mercier J. Balance of carbohydrate and lipid utilization during exercise: the" crossover" concept. J Appl Physiol 1994;76(6):2253-61.
37. Hamilton JA, Civelek VN, Kamp F, Tornheim K, Corkey BE. Changes in internal pH caused by movement of fatty acids into and out of clonal pancreatic beta-cells. J Biol Chem 1994;269(33):20852-6.
38. Holloszy JO, Kohrt WM, Hansen PA. The regulation of carbohydrate and fat metabolism during and after exercise. Front Biosci 1998;15:1011-27.

39. Hulston CJ, Venables MC, Mann CH, Martin C, Philp A, Baar K et al. Training with low muscle glycogen enhances fat metabolism in well-trained cyclists. Med Sci Sports Exerc 2010;42:2046-55.

40. Chenevière X, Borrani F, Droz D, Gojanovic B, Malatesta D. Effects of 2 different prior endurance exercises on whole-body fat oxidation kinetics: light vs. heavy exercise. Appl Physiol Nutr Metab 2012;37(5):955-64.

41. Børsheim E, Bahr R, Høstmark AT, Knardahl S. Effect of β-adrenoceptor blockade on postexercise oxygen consumption and triglyceride/fatty acid cycling. Metabolism 1998;47(4):439-48.

42. Livesey G, Elia M. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. Am J Clin Nutr 1988;47(4):608-28.

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TITLES OF TABLES

Table I. - Markers of muscle damage, and cardiorespiratory responses to low-intensity cycling, 12 h following downhill running in normal (NORM) and lowered muscle glycogen (LOW) conditions.

TITLES OF FIGURES

Figure 1. - Schematic of the experimental design.

Figure 2. - Respiratory exchange ratio (RER) during constant-load cycling performed before and after downhill running in normal (NORM) (A) and lowered glycogen (LOW) (B) conditions. Values are presented as mean ± SD; n = 11, one participant was unable to attain steady-state. * Significant pre-post downhill difference. Data refer to 4 to 10 min due to duration to attain steady-state.

Figure 3. - Fat oxidation during constant-load cycling performed before and after downhill running in normal (NORM) (A) and lowered glycogen (LOW) (B) conditions. Values are presented as mean ± SD; n = 11, one participant was unable to attain steady-state. * Significant pre-post downhill difference, $P < 0.05$. Data refer to 4 to 10 min due to duration to attain steady-state.

Figure 4. - Carbohydrate oxidation during constant-load cycling performed before and after downhill running in normal (NORM) (A) and lowered glycogen (LOW) (B) conditions. Values are presented as mean ± SD; n = 11, one participant was unable to attain steady-state. * Significant pre-post downhill difference, $P < 0.05$. Data refer to 4 to 10 min due to duration to attain steady-state.