The effect of glycogen reduction on cardiorespiratory and metabolic responses during downhill running

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Abstract

Purpose

Exercise-induced muscle damage and lowered glycogen are common during heavy training periods, and may prolong recovery. We examined the effects of lowered glycogen on cardiorespiratory, metabolic and perceptual responses to downhill running.

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Methods

Twelve men performed two downhill runs (−12 % gradient, 12.1 ± 1.1 km h^{-1}) separated by 6 weeks, under normal (NORM) and reduced glycogen (RED) conditions in a crossover design. For RED, participants performed exhaustive cycling at 60 % $\dot{V}O_{2max}$ power (95 ± 13 min) in the evening, and the next morning completed a downhill run comprising of five stages of 8 min running, with 2 min recovery (1 % gradient, 8 km h^{-1}) between each stage. Expired gas, heart rate, rating of perceived exertion (RPE) and blood lactate (bLa) and glucose were measured for each stage.

Results

Blood glucose ($P < 0.05$) and respiratory exchange ratio ($P < 0.01$) were lower in RED, than NORM, throughout the downhill run. RED demonstrated higher bLa until stage Four ($P < 0.05$), and RPE for stages Two and Five ($P < 0.05$).Ventilatory equivalent of carbon dioxide output ($\dot{V}_E/\dot{V}\mathrm{CO}_2$) was higher for stages One ($P < 0.01$), Two and Five ($P < 0.05$), and oxygen uptake ($\dot{V}_\mathrm{E}/\dot{V}\mathrm{O}_2$) was lower for stages Three and Four ($P < 0.05$) for RED.

Conclusions

Downhill running with reduced glycogen, elevated fat oxidation and bLa response, and, in part, increased effort perception. The alterations in $\dot{V}_\mathrm{E}/\dot{V}\mathrm{O}_2$ and bLa may suggest that carbon dioxide removal was somewhat impaired.

Keywords

Muscle damage
Glycogen
Exercise metabolism
Eccentric exercise
Effort perception
Respiration

Abbreviations

bLa	Blood lactate
DOMS	Delayed onset muscle soreness
HR	Heart rate
NORM	Normal glycogen
RED	Reduced glycogen
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
$\dot{V}\mathrm{CO}_2$	Carbon dioxide output
$\dot{V}_\mathrm{E}$	Minute ventilation

$\dot{V}_E/\dot{V}CO_2$	Ventilatory equivalent of carbon dioxide
$\dot{V}_E/\dot{V}O_2$	Ventilatory equivalent of oxygen uptake
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake

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Introduction

Prolonged, intense or unaccustomed exercise is known to induce muscle damage, leading to force loss, muscle soreness (Clarkson and Sayers 1999) and impaired glucose metabolism (Asp et al. 1995; Krishnan et al. 2003). After prolonged eccentric exercise, glycogen stores remain depleted up to 10 days (O'Reilly et al. 1987). It appears that eccentric-biased exercise not only disrupts muscle glycogen uptake and resynthesis, but does so in a delayed manner (Widrick et al. 1992). Altered glucose metabolism is known to result from exercise-induced insulin resistance, as reflected by decreased fat oxidation and preserved carbohydrate oxidation at rest in young men (Krishnan et al. 2003).

Earlier studies examining glycogen depletion had participants perform submaximal, exhaustive cycling (between 85 and 135 min) in the evening, followed by an overnight fast (Hughes et al. 1982; Segal and Brooks 1979). During moderate and heavy exercise the next morning, attenuated responses were shown for blood lactate (bLa), carbon dioxide output ($\dot{V}CO_2$) (Segal and Brooks 1979), respiratory exchange ratio (RER) and the ventilatory equivalent of oxygen uptake ($\dot{V}_E/\dot{V}O_2$) (Hughes et al. 1982). Glycogen depletion protocols have also been used to manipulate muscle fibre recruitment patterns prior to moderate-intensity exercise (Krustrup et al. 2004). Selectively depleting slow-twitch fibres resulted in enhanced fast-twitch fibre recruitment and increase oxygen uptake ($\dot{V}O_2$) during subsequent 50 % maximal oxygen uptake ($\dot{V}O_{2max}$) cycling. However, bLa accumulation was unchanged. It is also plausible that reducing muscle glycogen may increase fat oxidation in slow-twitch fibres for subsequent exercise. For example, lowering slow-twitch fibre glycogen content prior to heavy-intensity exercise has been shown to increase $\dot{V}O_2$ and fat oxidation, without altering $\dot{V}O_2$ kinetics or fibre recruitment patterns (Bouckaert et al. 2004). These authors reported respiratory and metabolic measures for concentric-biased exercise with lowered muscle glycogen, yet none have examined responses during eccentric-biased exercise with lowered glycogen.

Exercise-induced muscle damage and lowered glycogen are common during intense training periods. Muscle damage from downhill running has been shown to increase $\dot{V}O_2$, perceived exertion (Baumann et al. 2014), bLa and RER responses of young men undergoing subsequent level running (Hughes et al. 1982). Downhill running involves the knee extensors undergoing repetitive, intense lengthening contractions to decelerate the centre of mass, through a large range of motion (Eston et al. 1995). In this regard, downhill running represents a valid exercise mode to induce muscle damage that has particular application for endurance athletes, such as marathon and cross-country runners who experience trauma from surface impact and the changing terrain. As such, it is important to understand how a bout of exercise-inducing muscle damage is influenced by pre-exercise glycogen stores. Glycogen depletion appears to attenuate respiratory and metabolic responses to concentric exercise (Hughes et al. 1982; Segal and Brooks 1979). However, due to different physiological demands, the same cannot be expected for eccentric aerobic exercise. Downhill running incurs lower cardiovascular (Meyer et al. 2002

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) and energetic cost, in comparison to concentric exercise (Minetti et al. 2002), but does impair glucose metabolism after the event. Performing eccentric-biased, aerobic exercise with reduced glycogen, may promote our understanding of the role that substrate availability plays in the degenerative and regenerative processes of healthy, functioning muscle. Based upon the abovementioned findings, our working hypothesis states that pre-exercise glycogen reduction would increase bLa and cardiorespiratory responses for, and incurred damage from, subsequent muscle-damaging exercise. Therefore, the study aim was to investigate the effects of reduced muscle glycogen on the cardiorespiratory, metabolic and perceptual responses to muscle-damaging, downhill running in young, healthy men.

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Method

Participants

Twelve healthy men (Table 1) provided written informed consent for study participation. Sample size estimation was based on maximal isometric force loss (−15 %, α level = 0.05, power = 0.80) (Faul et al. 2007). Volunteers were recreationally active and had no history of structured resistance and/or regular running training. As confirmed by a medical history questionnaire, all were free from joint and musculoskeletal injury. The research protocol was approved by the University of Chichester Research Ethics Committee and conducted in accordance with the Helsinki Declaration.

Table 1

Participant characteristics ($n = 12$)

	Mean ± SD
Age (years)	23 ± 4
Height (cm)	179 ± 5
Body mass (kg)	76.6 ± 9.8
BMI (kg m^{-2})	23.9 ± 2.7
Body fat (%)	14.4 ± 3.8
$\dot{V}O_{2\max}$ (L $\min^{-1}$)	4.1 ± 1.0
$\dot{V}O_{2\max}$ (mL kg^{-1} $\min^{-1}$)	54 ± 9
Maximal workload (W)	314 ± 57
Glycogen reduction cycling workload (W)	181 ± 40
Downhill running speed (km h^{-1})	12.1 ± 1.1
Training time per week (h)	5.8 ± 3.3
Training sessions per week	4 ± 2

BMI body mass index, $\dot{V}O_{2\max}$ maximal ~~rate of~~ oxygen uptake

Experimental design

Cardiorespiratory responses were measured during two, downhill treadmill runs under (1) normal glycogen (NORM), and (2) reduced glycogen (RED) conditions. A randomised cross-over design was used, with each downhill run separated by at least 6 weeks. Blind selection was used to randomly allocate run order (NORM first, $n = 5$; RED first, $n = 7$), and only the first run was preceded by familiarisations. A repeat bout of the downhill running protocol has been shown not to affect knee extensor strength in a similar cohort 5 weeks later (Eston et al. 2000). Similarly, we found no order effect between downhill runs for maximal force loss ($P = 0.12$, $d = 0.11$). The NORM involved a resting evening session, followed by a morning run; RED involved an exhaustive, cycling exercise evening session, followed by a morning downhill run. Instruction was given to arrive hydrated, having abstained from heavy physical activity 48 h prior to the evening testing session. Aside from water ad libitum, food was not permitted until after the downhill run the next morning. Dietary manipulation required participants to arrive for the evening visits 3 h after their final meal (similar blood glucose, $P > 0.05$), having not consumed caffeine in the preceding 12 h. Habitual diet was maintained and self-recorded from 48 h prior to the first experimental condition, up to 48 h after the downhill run. Food records were checked by the investigator upon each visit and prescribed for the subsequent condition, as was physical activity, which was requested to remain low, according to the International Physical Activity Questionnaire (Craig et al. 2003).

Baseline measures

Familiarisation one involved anthropometric and resting measurements, followed by a maximal, incremental cycling test to prescribe intensity for glycogen reduction cycling. The cycling test was performed on an electronically controlled ergometer (Excalibur Sport 925900, Lode, Groningen, The Netherlands), with participants maintaining a ~75 rpm cadence at 50 W for 3 min, and thereafter 10 W increments every 20 s until volitional exhaustion (Osborne and Schneider 2006). A portable metabolic cart (Cosmed K4b^2, Rome, Italy) sampled expired gas breath by breath to determine $\dot{V}O_{2max}$, which was used to prescribe individual workload for glycogen reduction cycling.

Familiarisation two occurred ~48 h after the first, and ~48 h before the initial, evening experimental condition. A submaximal, incremental running test was performed to establish downhill running speed. Heart rate (HR), bLa and glucose were sampled after a 5 min seated rest, then participants warmed up for 5 min at 8 km h^{-1} (1 % gradient) on a pre-calibrated motorised treadmill (Pulsar, h/p/cosmos Sports and Medical GmbH, Germany). The test started at the warm-up speed, which was increased by 1 km h^{-1} every 4 min, until (1) eight stages, or (2) volitional exhaustion was reached. Expired gas was collected in the final 60 s of each stage with 200 L Douglas bags (Cranlea and Co., Birmingham, UK); HR (Polar Electro Oy, Kempele, Finland), rating of perceived exertion (RPE) (Borg 1998), and a fingertip capillary blood sample were measured in the final 30 s. Capillary blood (25 µL) was sampled from the index finger into EDTA-coated microvettes (~~SarstedtAktiengesellschaft~~Sarstedt Aktiengesellschaft and Co., Nümbrecht, Germany). Blood samples were analysed immediately for glucose and bLa concentrations using a pre-calibrated analyser (2300 STAT Plus™ analyser, YSI Life Sciences, Yellow Springs, USA), and then software (Newell et al. 2007) was used to estimate individual lactate threshold to prescribe downhill running speed.

Glycogen reduction cycling protocol

Participants reported to the laboratory in the evening (between 1905 and 1950 hours), 3 h after a light meal, to perform a preliminary cycling bout to lower muscle glycogen. After a 10 min warm up (~75 rpm at 50 % $\dot{V}O_{2\max}$), participants cycled at 60 % $\dot{V}O_{2\max}$ until volitional exhaustion (mean time, 95 ± 13 min), as confirmed by an inability to maintain a 50 rpm cadence. Expired gas, HR, RPE and fingertip blood were measured every 20 min (Table 2). The protocol was modified from previous studies (Osborne and Schneider 2006; Thomson et al. 1979), having used biopsy to confirm muscle fibre glycogen depletion (slow twitch, 95 %; fast twitch, 70 %) (Thomson et al. 1979). For NORM, participants reported to the laboratory in the evening, provided resting measurements after a 5 min rest, and then remained seated in a dimly-lit room for a period of 2 h. Arrival times and dietary instruction were matched between the randomised conditions.

Table 2

Mean physiological responses to glycogen reduction cycling

	20 min (*n* = 12)	**40 min (*n* = 12)**	**60 min (*n* = 12)**	**80 min (*n* = 12)**	**100 min (*n* = 7)**
Workload (*W*)	181 ± 32	174 ± 29	165 ± 28	163 ± 28	170 ± 34
Cadence					

(rpm)	74 ± 2	73 ± 2	73 ± 2	73 ± 2	79 ± 1
$\dot{V}_E$ (L min^{-1})	77.9 ± 14.2	78.3 ± 16.0	76.6 ± 16.8	80.8 ± 17.4	73.5 ± 14
$\dot{V}O_2$ (L min^{-1})	2.56 ± 0.38	2.59 ± 0.49	2.57 ± 0.49	2.56 ± 0.76	2.66 ± 0.
$\dot{V}CO_2$ (L min^{-1})	2.44 ± 0.21	2.4 ± 0.28	2.32 ± 0.24	2.36 ± 0.27	2.21 ± 0.
$\dot{V}O_2$ (mL kg min^{-1})	33.7 ± 6.8	34.1 ± 8.2	33.8 ± 8.4	33.8 ± 12.1	35.8 ± 11
FeO_2 (%)	16.8 ± 1.0	16.8 ± 1.0	16.8 ± 1.1	17.0 ± 1.2	16.6 ± 1.
$FeCO_2$ (%)	3.9 ± 0.6	3.8 ± 0.6	3.8 ± 0.6	3.7 ± 0.5	3.7 ± 0.5
$\dot{V}_E/\dot{V}O_2$ (L min^{-1})	31.1 ± 8.0	31.3 ± 9.1	30.8 ± 9.0	34.3 ± 14.5	29.7 ± 10
$\dot{V}_E/\dot{V}CO_2$ (L min^{-1})	31.8 ± 4.4	32.4 ± 4.8	32.8 ± 5.4	33.9 ± 4.3	33.3 ± 4.
RER	0.95 ± 0.12	0.94 ± 0.15	0.92 ± 0.15	0.91 ± 0.16	0.88 ± 0.
bLa (mmol L^{-1})	3.77 ± 1.56	2.62 ± 0.83	2.67 ± 0.63	2.84 ± 1.08	2.26 ± 1.
Glucose (mmol L^{-1})	3.83 ± 0.40	3.37 ± 0.29	3.39 ± 0.39	3.18 ± 0.49	2.96 ± 0.
HR (b min^{-1})	162 ± 9	162 ± 9	162 ± 6	162 ± 10	157 ± 15
RPE	13.4 ± 1.5	15.5 ± 1.2	16.6 ± 1.5	18.1 ± 1.1	18.4 ± 0.

Values are mean ± SD

$\dot{V}_E$ minute ventilation, $\dot{V}O_2$ oxygen uptake, $\dot{V}CO_2$ carbon dioxide production, Fe
of expired oxygen, $FeCO_2$ fraction of expired carbon dioxide, $\dot{V}_E/\dot{V}O_2$ ventilator
of oxygen uptake, $\dot{V}_E/\dot{V}CO_2$ ventilatory equivalent of carbon dioxide production,
respiratory exchange ratio, *bLa* blood lactate, *HR* heart rate, *RPE* rating of perceiv
n indicates number of subjects at timepoint of the glycogen reduction cycling prot

Downhill running

Participants attended the laboratory in the morning (between 0650 and 0715 hours), to perform downhill running (−12 % gradient) at individual lactate threshold speed (12.1 ± 1.1 km h^{-1}). After a 5 min warm up (1 % gradient at 8 km h^{-1}), participants performed five, 8 min stages of downhill running, separated by 2 min recovery periods (1 % gradient at

8 km h^{-1}). Previously used with young individuals, at a fixed running speed (11.3 km h^{-1}), the protocol was effective in inducing knee extensor soreness and maximal force loss up to 72 h later (Eston et al. 2000). Expired gas was sampled in the final 60 s of each stage, and HR, RPE and fingertip blood in the final 30 s. Between conditions, the same footwear and treadmill were used in a temperature controlled laboratory (19–21 °C).

Knee extensor soreness and maximal force were measured on a custom-built, strength-testing chair with hip and knee angle at 1.57 rad, before and after downhill running (at 0 and 48 h). Soreness was assessed using a visual analogue scale (0 = not at all sore; 10 = extremely sore), with the muscle-belly palpated until enough pressure was exerted to blanch the fingernail. Force was measured during three separate, 3–5 s maximal isometric contractions (2 min rests), with verbal encouragement and force–time feedback provided by the computer monitor.

Statistical analysis

A paired sample *t* test was used to examine whether there was an order effect by analysis of maximal force loss and soreness at 48 h for bout 1 and 2. A two-way repeated measures ANOVA was used to compare physiological responses during each stage of downhill running, and maximal force loss, between NORM and RED conditions. Differences were located using pre-planned *t* tests with a Bonferroni correction used to control for multiple comparisons. Where assumptions of sphericity were violated, a Greenhouse-Geisser correction was applied. Friedman tests were used on non-parametric: muscle soreness, RPE, $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ data, with Wilcoxon signed-rank tests locating individual stage differences. Effect sizes (Cohen's *d*) were calculated for all significant post hoc tests of parameters recorded during downhill running. These were interpreted as small (0.2–0.3), moderate (0.31–0.79) and large (≥0.8) effect size (Thomas et al. 1991). Data are presented as mean ± SD, with an alpha level of <0.05.

Results

Markers of muscle damage

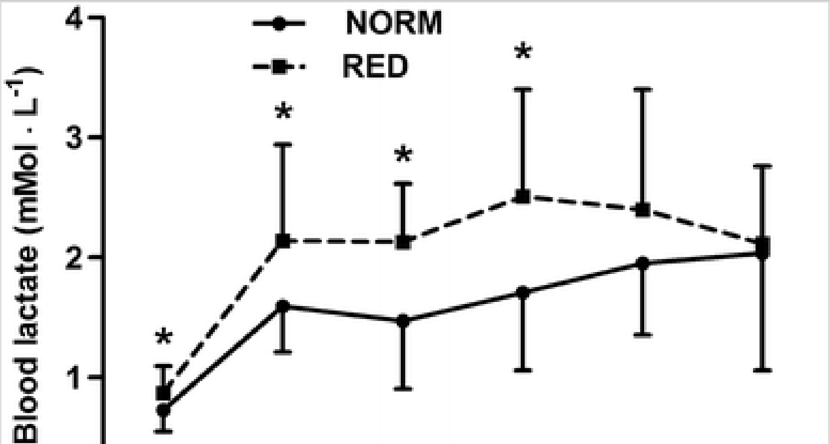
Our experimental design did not show an order effect as post-exercise, the force loss at 48 h (bout 1: −19 ± 1 %, bout 2: −15 ± 18 %, $P = 0.43$), and muscle soreness at 48 h (bout 1: 3.6 ± 1.6, bout 2: 4.5 ± 2.0, $P = 0.12$) were similar. Maximal isometric force loss from downhill running was similar between conditions ($P > 0.05$), immediately after (NORM, −24 ± 15 %; RED, −27 ± 10 %) but higher at 48 h for RED (NORM, −12 ± 14 %; RED, −23 ± 14 %, $P < 0.05$). Muscle soreness showed a significant effect; soreness was higher for RED (3.9 ± 1.8), than NORM (3.0 ± 1.2; $P < 0.05$) immediately after, but similar 48 h after (NORM, 3.8 ± 1.2; RED, 4.4 ± 2.3, $P = 0.37$).

Blood lactate concentration

During downhill running, bLa was significantly different between conditions ($P < 0.05$) with no interaction effect. Resting bLa was moderately higher for RED (0.84 ± 0.23 mmol L^{-1}), than for NORM (0.71 ± 0.23 mmol L^{-1}, $P < 0.05$, $d = 0.62$; Fig. 1). After stage One, bLa was 2.14 ± 0.93 mmol L^{-1} for RED, and 1.59 ± 0.46 mmol L^{-1} for NORM ($P < 0.05$, $d = 0.75$, moderate effect size). From stages Two to Three, bLa had increased from 2.13 ± 0.57 to 2.54 ± 1.04 mmol L^{-1} for RED, and from 1.47 ± 0.55 ($d = 1.18$, large effect size) to 1.61 ± 0.53 mmol L^{-1} ($d = 1.14$, large effect size) for NORM respectively. Thereafter, mean bLa was similar between RED and NORM (Fig. 1).

Fig. 1

Blood lactate concentrations (bLa) at rest, and during downhill running with normal (NORM) and reduced (RED) glycogen. Values are mean ± SD. *Significant difference between conditions, $P < 0.05$

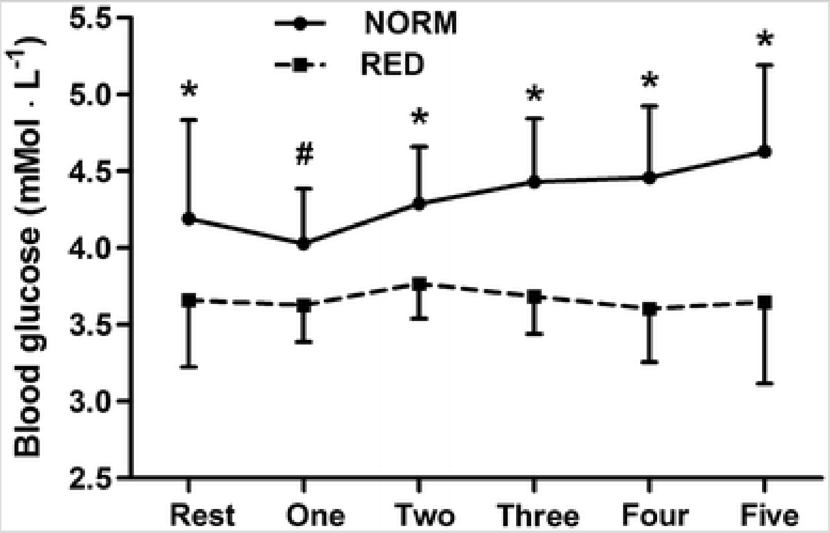


Blood glucose concentration

During downhill running, blood glucose concentration showed a significant condition–time interaction effect ($P = 0.03$). Pre-downhill, glucose (Fig. 2) was lower in RED than in NORM (3.66 ± 0.44 mmol L^{-1}, NORM, 4.19 ± 0.64 mmol L^{-1}; $P < 0.01$, $d = 1.03$, large effect size). Blood glucose remained lower in RED, than in NORM, from stages One (3.63 ± 0.28 mmol L^{-1}; NORM, 4.03 ± 0.42 mmol L^{-1}, $P < 0.05$, $d = 1.33$, large effect size) to Five (3.65 ± 0.53 mmol L^{-1}; NORM, 4.63 ± 0.56 mmol L^{-1}, $P < 0.01$, $d = 1.80$, large effect size) of downhill running.

Fig. 2

Blood glucose concentrations at rest, and during downhill running with normal (*NORM*) and reduced (*RED*) glycogen. Values are mean ± SD. *Significant difference between conditions, $P < 0.05$. $^{\#}$ Trend, $P = 0.08$



Cardiorespiratory and perceptual responses

There were no consistent differences between conditions for minute ventilation ($\dot{V}_E$), $\dot{V}O_2$, $\dot{V}CO_2$ or HR ($P > 0.05$). During running, $\dot{V}_E$ was similar, increasing by 4.8 L min^{-1} for RED ($d = 0.40$) and 6.2 L min^{-1} for NORM by stage Five ($P < 0.01$, $d = 0.57$, moderate effect size; Table 3). Similarly, $\dot{V}O_2$ and $\dot{V}CO_2$ increased by stage Five in both conditions ($P < 0.05$; Table 3). Mean $\dot{V}_E/\dot{V}O_2$ was different between conditions ($P < 0.05$), lower for RED, than NORM, at stages Three ($P < 0.05$, $d = 0.49$, moderate effect size) and Four ($P < 0.05$, $d = 0.70$, moderate effect size; Table 3). Mean $\dot{V}_E/\dot{V}CO_2$ was significantly higher for RED, than NORM ($P < 0.05$), during stages One (2.0 L min^{-1}, $P < 0.01$,

$d = 0.65$, moderate effect size), Two (1.4 L min^{-1}, $P < 0.05$, $d = 0.51$, moderate effect size) and Five (1.0 L min^{-1}, $P < 0.05$, $d = 0.30$, small effect size). For RED, HR appeared higher than NORM in stage Two ($P < 0.05$, $d = 0.20$, small effect size), and was higher in stage Five, than stage One in both conditions (Table 3) with no interaction effect. RPE was significantly ($P < 0.001$) higher in stages Two ($P < 0.05$, $d = 0.58$, moderate effect size) and Five ($P < 0.05$, $d = 0.36$, moderate effect size) with no interaction effect.

Table 3

Cardiorespiratory, metabolic and perceptual responses during downhill running in reduced (RED) glycogen conditions

	Stage One		**Stage Two**		**Stage**
	NORM	**RED**	**NORM**	**RED**	**NOR**
$\dot{V}_E$ (L min^{-1})	52.7 ± 6.6	54.9 ± 8.3	53.9 ± 8.5	55.7 ± 9.8	59.3 ±
$\dot{V}O_2$ (L min^{-1})	2.11 ± 0.37	2.18 ± 0.36	2.23 ± 0.38	2.33 ± 0.32	2.32 ±
$\dot{V}CO_2$ (L min^{-1})	1.95 ± 0.35	1.90 ± 0.29	2.02 ± 0.36	1.99 ± 0.32	2.17 ±
$\dot{V}O_2$ (mL kg min^{-1})	27.6 ± 4.0	28.3 ± 3.1	29.0 ± 3.7	30.2 ± 2.2*	30.2 ±
$\dot{V}_E/\dot{V}O_2$ (L min^{-1})	30.8 ± 3.5	30.7 ± 3.2	29.7 ± 3.7	29.1 ± 3.1	31.3 ±
$\dot{V}_E/\dot{V}CO_2$ (L min^{-1})	33.2 ± 3.4	35.2 ± 2.6*	32.7 ± 3.2	34.1 ± 2.5*	33.6 ±
RER	0.93 ± 0.04	0.87 ± 0.05*	0.91 ± 0.05	0.85 ± 0.06*	0.93 ±
HR (b min^{-1})	148 ± 15	154 ± 16	154 ± 16	158 ± 17*	160 ±
RPE	11.0 ± 2.0	11.8 ± 1.5	13.1 ± 1.9	14.2 ± 1.9*	14.8 ±

	Stage Four		**Stage Five**		***P* val**
	NORM	**RED**	**NORM**	**RED**	
$\dot{V}_E$ (L min^{-1})	57.0 ± 10.9	55.1 ± 8.4	58.9 ± 10.8[#]	59.7 ± 12.1[#]	0.081
$\dot{V}O_2$ (L min^{-1})	2.30 ± 0.50	2.36 ± 0.35	2.37 ± 0.50[#]	2.43 ± 0.36[#]	0.571
$\dot{V}CO_2$ (L min					

$^{-1}$)	2.08 ± 0.45	1.98 ± 0.32	2.12 ± 0.45$^{\#}$	2.06 ± 0.38$^{\#}$	0.439
$\dot{V}O_2$ (mL kg min^{-1})	29.9 ± 4.6	30.5 ± 2.4	30.7 ± 4.4$^{\#}$	31.4 ± 2.6	0.568
$\dot{V}_E/\dot{V}O_2$ (L min^{-1})	30.5 ± 3.8	28.3 ± 2.1*	30.6 ± 4.0	29.6 ± 3.0	0.027
$\dot{V}_E/\dot{V}CO_2$ (L min^{-1})	33.6 ± 3.2	34.0 ± 2.0	34.2 ± 3.9	35.2 ± 2.1*	0.013
RER	0.91 ± 0.05	0.83 ± 0.05*	0.89 ± 0.04$^{\#}$	0.84 ± 0.06*	0.000
HR (b min^{-1})	165 ± 17	165 ± 16	166 ± 17$^{\#}$	167 ± 16$^{\#}$	0.136
RPE	15.5 ± 1.9	15.8 ± 1.9	16.1 ± 2.1$^{\#}$	16.8 ± 1.8$^{*,\#}$	0.021

Values are mean ± SD

$\dot{V}_E$ minute ventilation, $\dot{V}O_2$ oxygen uptake, $\dot{V}CO_2$ carbon dioxide production, $\dot{V}$ equivalent of oxygen uptake, $\dot{V}_E/\dot{V}CO_2$ ventilatory equivalent of carbon dioxide respiratory exchange ratio, *HR* heart rate, *RPE* rating of perceived exertion

*-Difference between conditions, *t* test

[#]Difference between stages One and Five, *t* test, $P < 0.05$

[a]Two-way ANOVA *P* value

[b]Friedman test *P* value

Respiratory exchange ratio

RER during downhill running was significantly different between conditions ($P < 0.05$). For RED, the RER was lower from stage One (0.87 ± 0.05; NORM, 0.93 ± 0.04; $P < 0.01$, $d = 1.33$, large effect size) to stage Five (0.84 ± 0.06; NORM, 0.89 ± 0.04; $P < 0.01$, $d = 0.98$, large effect size), compared to NORM (Table 3). In NORM, RER was lower in stage Five compared to stage One (Table 3).

Discussion

Our main finding in this study was that performing a bout of aerobic, eccentric exercise with lowered glycogen, increased fat oxidation, bLa response, and effort perception, when compared to an eccentric bout with normal glycogen. Cardiorespiratory responses showed little difference between conditions; however, during the RED downhill run, either $\dot{V}_E/\dot{V}CO_2$ was elevated or $\dot{V}_E/\dot{V}O_2$ was reduced. Increased bLa and altered ventilatory responses may reflect reliance upon type II fibres when performing muscle-damaging exercise with lowered muscle glycogen.

The present study used a cycling protocol that had previously been shown by Thomson et al. (1979) to cause 95 % glycogen depletion in type I fibres in healthy men after 2 h. Assuming similar type I glycogen depletion occurred in this study, our participants would be expected to commence the RED downhill run, recruiting predominantly type II fibres, which are more prone to eccentric-induced muscle damage (Fridén et al. 1983). This would agree with an earlier study (Krustrup et al. 2004), in which slow-twitch fibre depletion led to greater fast-twitch fibre recruitment and $\dot{V}O_2$ for subsequent cycling exercise. We found similar $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$and HR responses between conditions, from stage One to Five. This may be attributed to the relatively low intensity of downhill running. For example, participants exercised between 62 and 65 % $\dot{V}O_{2max}$ for glycogen reducing cycling, and between 51 and 59 % $\dot{V}O_{2max}$ for downhill running. Therefore, the running intensity may not have been high enough to evoke distinguishable ventilatory responses between NORM and RED conditions. An eccentric protocol using higher intensities is likely to lead to greater muscle damage and delayed onset of muscle soreness (DOMS) (Paschalis et al. 2005). In this study we intended to investigate a whole-body eccentric-biased exercise common to both: day-to-day occupational activities, such as walking and stair descent, and endurance activities, such as marathon and cross-country running.

Previously, pre-exercise glycogen depletion has been shown to increase $\dot{V}O_2$, without altering $\dot{V}O_2$ kinetics for cycling (Bouckaert et al. 2004). After 9 min of high intensity cycling (85 % $\dot{V}O_{2peak}$ power), bLa increased to 8.1 mmol L^{-1} for normal glycogen, and 6.9 mmol L^{-1} for depleted glycogen. The end-of-exercise bLa values in this study were 1.98 mmol L^{-1} for NORM and 2.14 mmol L^{-1} for RED. These differences can be attributed to the exercise mode, intensity and duration. In particular, we found no change in $\dot{V}O_2$ until stage Five of downhill running. Glycogen reduction protocols were similar between studies, but Bouckaert et al. (2004) used high intensity, short duration, concentric-biased exercise, whereas we used moderate intensity, prolonged, eccentric-biased exercise. In order to induce similar rates of $\dot{V}O_2$ increase between contraction types, intensity must be high for eccentric exercise and low for concentric exercise (Perrey et al. 2001). As such, the $\dot{V}O_2$ response to high intensity, concentric exercise, would be expected to be greater than that of moderate intensity, eccentric-biased downhill running. Using similar glycogen depleting exercise, others have reported increased $\dot{V}_E$, and decreased $\dot{V}CO_2$, for subsequent cycling exercise at moderate (55 % $\dot{V}O_{2max}$) and heavy (95 % $\dot{V}O_{2max}$) intensities (Segal and Brooks 1979). As expected, in a glycogen depleted state, heavy exercise was shown to elicit greater change in $\dot{V}_E$ and $\dot{V}CO_2$, than moderate exercise. Interestingly, lactate response was unchanged for heavy exercise, but lower for moderate exercise. Unusual under normal circumstances, these findings were attributed to the remaining glycogen in type II fibres. For example, when exercising with depleted glycogen, the reduced glucose concentrations are typically accompanied by reduced lactate concentrations (Bouckaert et al. 2004; Hughes et al. 1982) due to a shift from glycolytic metabolism. Therefore, exercising with RED would cause lower circulating glucose and bLa. The current study showed reduced glucose, but increased bLa up to stage Four of downhill running with RED, compared to NORM. This may be due to greater muscle mass used for downhill running, and type II fibre recruitment, when compared to cycling exercise. Similar bLa from stage Four may be explained by RED involving higher type II recruitment, anaerobic glycolysis, and therefore bLa accumulation (stages One to Three), until further glycogen decrease, leading to a glycolytic to oxidative metabolic shift (stages Four to Five). Conversely, glycogen availability allowed sustained glycolysis for NORM, as glucose and lactate seemed to increase progressively throughout running. Elsewhere, Scott et al. (2003) found that eccentric contractions did not affect bLa during a subsequent bout of moderate intensity level running (~67 % $\dot{V}O_{2max}$). These authors concluded that predominant type II fibre damage would result in type I fibre recruitment for subsequent running. Type II fibre predominance (and therefore high glycogen utilisation) in our protocol was likely evident as NORM downhill running caused (1) a concomitant rise in $\dot{V}O_2$ and lactate, and (2) an RER above 0.89 throughout. Our downhill running intensity was prescribed from individual lactate threshold, therefore if glycogen reduction cycling depleted mainly type I fibres (Thomson et al. 1979), our participants would be expected to commence downhill running using type II fibres. Downhill running also involves a large component of eccentric knee extensions, which would cause preferential type II fibre damage (Fridén et al. 1983).

Muscle soreness was not measured during downhill running, but effort perception increased from the first (~11.4, light exertion) to final stages (~16.5, hard/very hard exertion) for both conditions. Effort perception was greater for RED for stages Two and Five, which, in part, agrees with higher RPE for 85 % $\dot{V}O_{2peak}$ cycling with glycogen depletion (Bouckaert et al. 2004). For submaximal running, DOMS is known to alter gait and increase energy cost (Braun and Dutto 2003). We found increased RPE during RED downhill running, suggesting greater perceived effort in a glycogen reduced state. This finding may reflect an earlier emergence of muscle damage during the RED downhill run. For example, Braun and Dutto (2003) suggested that the greatest damage occurs in the latter stages of downhill running due to altered gait kinematics. We are uncertain that glycogen reduction influenced running gait, and therefore muscle damage, in this study as biomechanical parameters were not examined. However, the potential effect of glycogen reduction on running biomechanics warrants further investigation, particularly for those working with endurance runners.

Performing heavy cycling exercise (85 % $\dot{V}O_{2max}$) with glycogen depletion has been shown to increase $\dot{V}O_2$, and decrease HR and RER (Krustrup et al. 2004). We showed lower RER, and similar $\dot{V}O_2$ and HR for the RED downhill run, when compared to NORM. Decreased RER and decreased blood glucose concentration during the RED downhill run is indicative of greater fat oxidation. For a given concentric workload, increased fat metabolism in type I fibres elevates $\dot{V}O_2$, to a greater degree than carbohydrate metabolism (Vøllestad et al. 1984; Yamanaka et al. 2012) Whether this is true for eccentric exercise remains to be seen, yet lower RER provides an indirect measure of our glycogen reduction protocol, as fat oxidation rate is determined by carbohydrate availability (Holloszy et al. 1998).

For low to moderate exercise intensities, $\dot{V}_E/\dot{V}O_2$ remains stable. As intensity increases, ventilation rises disproportionately and uncouples from $\dot{V}O_2$, reflecting the ventilatory threshold. Beyond this threshold, the anaerobic metabolism is defined as an increase in $\dot{V}_E/\dot{V}O_2$ and lactate, without change in $\dot{V}_E/\dot{V}CO_2$ (Caiozzo et al. 1982). At high intensities, increased $\dot{V}_E/\dot{V}O_2$ suggests ventilation cannot satisfy muscle oxygen delivery, and increased $\dot{V}_E/\dot{V}CO_2$ suggests ventilation cannot remove excess carbon dioxide. Higher $\dot{V}_E/\dot{V}CO_2$ commencing the RED downhill run was supported by increased bLa. In agreement, increased $\dot{V}_E/\dot{V}CO_2$ and decreased $\dot{V}_E/\dot{V}O_2$ have been shown for incremental cycling exercise with depleted glycogen (Hughes et al. 1982). Elsewhere, increased $\dot{V}_E/\dot{V}O_2$ has been associated with greater effort perception when exercising with muscle damage (Twist and Eston 2009). Had RED resulted in greater hydrogen ion buffering during the downhill run, $\dot{V}CO_2$, and, in turn, ventilation would increase. Although $\dot{V}_E$ was similar between conditions, this would agree with higher initial bLa shown for RED downhill running. Ventilatory equivalent responses varied over running stages between conditions, partly due to fluctuations in $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$. For example, non-significantly higher $\dot{V}_E$, and lower $\dot{V}CO_2$, for RED (stages One, Two and Five) contributed to an increased $\dot{V}_E/\dot{V}CO_2$. Stabilised $\dot{V}_E$, with a non-significantly higher $\dot{V}O_2$, for RED (stages Three and Four) led to decreased $\dot{V}_E/\dot{V}O_2$.

Our protocol featured a recovery period between the glycogen reduction cycling and the downhill run of ~10 h. This interval allowed time for other confounding physiological disturbances, aside from glycogen reduction, associated with prolonged exercise (e.g., body temperature and hormonal alterations) to pass. Thus, for the downhill run we are confident that the effects of lowered muscle glycogen, and not the effects of other exercise-induced perturbations, were being observed. The majority of glycogen repletion occurs within 3 h post-exercise (Maehlum and Hermansen 1978). Muscle glycogen content could not be directly measured, however, the following provide evidence that glycogen stores were lowered for the RED condition. First, blood glucose concentrations were substantially lower before and during the downhill run. Second, the RER was greatly reduced throughout the RED run, suggesting greater fat oxidation. Finally, a glycogen reduction protocol was used, shown to deplete glycogen in the knee extensor muscles of healthy men (Thomson et al. 1979). Nevertheless, it needs to be acknowledged that the extent of glycogen depletion in the present study was based on a protocol with biopsy-confirmed glycogen levels. There is still the possibility that the extent of glycogen depletion in the present may differ from previous studies and it is a limitation that glycogen levels were not confirmed by direct biopsy observations.

This study demonstrated that downhill running performed with reduced muscle glycogen caused an attenuated blood glucose response, lowered RER, and in part, an increased blood lactate response. Cardiorespiratory responses were unchanged, yet ventilatory equivalents were somewhat altered, with glycogen reduction appearing to further stimulate carbon dioxide removal during downhill running. This is notable, given that relative exercise intensity was low and ventilatory equivalents were influenced by glycogen state, but not running duration. The alterations in $\dot{V}_E/\dot{V}O_2$ and bLa may suggest that carbon dioxide removal was somewhat impaired. Future work should determine whether lowered glycogen affects consistently the cardiorespiratory responses to more intense eccentric exercise. Evidence for initial type II recruitment during RED downhill running includes, (1) increased bLa (up to stage Three), and (2) increased $\dot{V}_E/\dot{V}CO_2$, and decreased $\dot{V}_E/\dot{V}O_2$. These findings have implications for recovery strategies of athletes performing new or heavy training, as well as demonstrating that glycogen reduction alters both ventilatory and metabolic responses to eccentric-biased exercise.

Conflict of interest

The authors declare no conflicts of interests.

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