

Glycogen utilisation during running: intensity, sex and muscle specific responses

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41 **Abstract**

42 **Purpose:** To quantify net glycogen utilisation in the vastus lateralis (VL) and
43 gastrocnemius (G) of male (n=11) and female (n=10) recreationally active runners
44 during three outdoor training sessions. **Methods:** After 2 days standardisation of
45 carbohydrate (CHO) intakes (6 g.kg⁻¹ body mass per day), glycogen was assessed
46 before and after 1) a 10-mile road run (10-mile) at lactate threshold, 2) 8 x 800 m track
47 intervals (8 x 800 m) at velocity at $\dot{V}O_{2max}$ and 3) 3 x 10 minute track intervals (3 x 10
48 min) at lactate turnpoint. **Results:** Resting glycogen concentration was lower in the G
49 of females compared with males (P<0.001) though no sex differences were apparent in
50 the VL (P=0.40). Within the G and VL of males, net glycogen utilisation differed
51 between training sessions where 10-mile was greater than both track sessions (all
52 comparisons, P<0.05). In contrast, net glycogen utilisation in females was not different
53 between training sessions in either muscle (all comparisons, P>0.05). Net glycogen
54 utilisation was greater in males than females in both VL (P=0.02) and G (P=0.07)
55 during the 10-mile road run. With the exception of males during the 3 x 10 min protocol
56 (P=0.28), greater absolute glycogen utilisation was observed in the G versus the VL
57 muscle in both males and females and during all training protocols (all comparisons,
58 P<0.05). **Conclusion:** Data demonstrate 1) prolonged steady state running necessitates
59 a greater glycogen requirement than shorter but higher intensity track running sessions,
60 2) females display evidence of reduced resting muscle glycogen concentration and net
61 muscle glycogen utilisation when compared with males and 3), net glycogen utilisation
62 is higher in the gastrocnemius muscle compared with the vastus lateralis.

63 **Keywords:** muscle glycogen, carbohydrate, training, gender

65 **Introduction**

66 Since the introduction of the muscle biopsy technique in the late 1960s (1), the
67 importance of muscle glycogen for augmenting exercise capacity and performance in
68 endurance events has been well documented. In addition to high endogenous
69 carbohydrate (CHO) availability, augmenting exogenous CHO availability (typically
70 via gels, drinks or bars) is also ergogenic to exercise performance (2), an effect likely
71 mediated by liver (3) and muscle glycogen sparing (4), maintaining plasma glucose and
72 CHO oxidation rates (5,6) and/or via direct effects on the central nervous system (7).
73 When taken together, nutritional guidelines for competitive endurance events
74 recommend sufficient CHO loading (e.g. 7-12 g/kg body mass depending on event
75 duration) to ensure elevated muscle and liver glycogen stores, as well as to consume
76 exogenous CHO when exercise duration is > 1 hour (8,9).

77 In contrast to competition, contemporary guidelines for training recognize the need to
78 adjust daily CHO intake according to the goal of enhancing training quality versus
79 creating a metabolic stimulus that may enhance training adaptation (8). In this regard,
80 the emergence of the “train-low” paradigm is based on the premise that periodically
81 completing selected training sessions with reduced CHO availability up-regulates acute
82 skeletal muscle cell signaling pathways (10) thereby leading to enhanced oxidative
83 adaptations of skeletal muscle (11,12,13) and potentially, improved exercise
84 performance and capacity (14,15). From a practical perspective, we recently
85 communicated the concept of CHO periodization according to the principle of fuel for
86 the work required, whereby CHO availability is manipulated day-by-day and meal-by-
87 meal in relation to the demands of the specific training session (16,17).

88 Despite the theoretical rationale for CHO periodisation strategies, practical application
89 is limited by the lack of data quantifying the glycogen cost associated with specific
90 training sessions. Indeed, despite over five decades of research examining glycogen
91 metabolism during exercise, the majority of data are based on laboratory protocols (e.g.
92 fasted exercise undertaken at a fixed relative intensity for a given duration, e.g. 1 h at
93 70% $\text{VO}_{2\text{max}}$) that may not always be applicable to the field-based training sessions.
94 For example, the oxygen cost of outdoor running is greater than running on a treadmill
95 (19) and the training intensities prescribed to athletic populations are typically anchored
96 to lactate threshold (as opposed to maximal oxygen uptake) and completed in the fed
97 state. Almost a decade ago, Burke et al. (2011) (9) highlighted that CHO guidelines
98 for athletic populations are not underpinned by direct knowledge of the glycogen cost
99 of real-life exercise programs, sentiments that were also communicated in 2016 (8) and
100 2018 (19). In the former paper, the authors suggested that to estimate the substrate
101 requirement of specific workouts, practitioners must rely on guesswork supported by
102 information obtained from consumer based activity / heart rate monitors and global
103 positioning systems. In the latter paper, the fuel costs, glycogen utilization rates and
104 associated CHO intake requirements of habitual training sessions was identified as a
105 targeted question for future research (19).

106 In addition to the specifics of the exercise protocol, differences in muscle group
107 examined (12,20) and sex-specific alterations in substrate metabolism (21) may also
108 affect the absolute glycogen utilisation associated with specific training sessions. To
109 our knowledge, however, such comparisons of muscle group, sex, and training intensity
110 have not yet been simultaneously examined within the same study. The paucity of data
111 from female participants was recognized by Devries et al. (2016) (22) though is much
112 stronger evidenced in the recent meta-analysis of Areta and Hopkins (2018) (23).

Indeed, of the 180 studies that assessed glycogen utilization in human skeletal muscle during exercise, less than 5% included female participants.

Accordingly, the aim of the present study was to quantify net glycogen utilization of the vastus lateralis and gastrocnemius muscles of recreationally active male and female runners during three types of training sessions considered representative of runners' habitual workouts. We deliberately chose recreationally active runners given that they comprise the largest running population and hence, our data may have greater practical relevance. To this end, we quantified glycogen use during a 10-mile road run conducted at lactate threshold, an 8 x 800 m track interval session (8 x 800 m) completed at velocity at $\dot{V}O_{2peak}$ and finally, a 3 x 10 minute (3 x 10 min) track interval session undertaken at a velocity corresponding to lactate turnpoint.

Methodology

Participants: After providing informed written consent, twenty-one competitive and recreational runners (11 males and 10 females) volunteered to take part in the study. Inclusion criteria consisted of a minimum of 3 years competitive running experience, habitually training ≥ 3 times per week, and 10 km race time ≤ 45 min for males and ≤ 50 min for females. Participants' anthropometric, training history and physiological profiles is displayed in Table 1. All procedures confirmed to the standards set by the *Declaration of Helsinki* and the study was approved by the NHS Research Authority of the United Kingdom (West Midlands, Black Country Research Ethics Committee, REC reference 15/WM/0428).

Design: In a randomised and repeated measures design, participants completed three sessions considered representative of those undertaken by runners competing in 10 km events (Spillsbury, personal communication, English Institute of Sport). The training sessions consisted of 1) a 10-mile road run (10-mile) undertaken at a velocity corresponding to lactate threshold, 2), an 8 x 800 m track interval session (8 x 800 m) competed at velocity at $\dot{V}O_{2peak}$ and 3), a 3 x 10 minute (3 x 10 min) track interval session undertaken at velocity corresponding to lactate turnpoint. A summary of each training session is also displayed in Table 2. In an attempt to compare with previously published data (24,25,26,27,28) and also considering that glycogen utilisation is lower during the luteal phase (24), we deliberately studied female participants (who self-reported) during the mid follicular phase (days 7 – 10). In this way, male participants had 7-10 days between trials whereas females had 28 days between trials. Female participants were eumenorrheic with a normal cycle length, and inclusion criteria included use of oral contraception (combined pill), diaphragm or intrauterine device (IUD). Muscle biopsies from both the vastus lateralis (VL) and gastrocnemius (G) muscles and venous blood samples were obtained immediately before and after completion of each training session. At 48 h prior to commencement of each training session, participants completed a standardised training session followed by standardised dietary intakes in an attempt to replicate pre-exercise muscle glycogen concentration between trials. All food was provided to the participants in pre-prepared packages having been prepared by a registered sports nutritionist (SENr) (Author 1).

Baseline assessments: The running velocity at which each participant completed the three training sessions was determined by completion of a 2-part incremental exercise test on a motorised treadmill (HP Cosmos, Germany) to establish lactate threshold, lactate turn-point and peak oxygen uptake ($\dot{V}O_{2peak}$). Participants reported to the

laboratory in a fasted state between 07:00 - 08:00 for an initial assessment of body composition via dual energy X-ray absorptiometry (DXA) (Hologic QDR Series, Discovery A, Bedford, MA, USA) according to the DXA best practice protocol (29). Participants were then provided with a standardised breakfast (2 g.kg⁻¹ body mass CHO, 25 g protein, 10 g fat) at 3 hours prior to commencing the incremental exercise test. To replicate outdoor running conditions (18), the test was commenced at 1% incline (at 8 and 10 km.h⁻¹ for females and males, respectively) and after a 10-minute self-selected warm-up. Oxygen uptake was measured continuously during exercise via breath-by-breath measurement using a CPX Ultima series online gas analysis system (Medgraphics, Minnesota, USA). The treadmill speed was increased by 1 km.h⁻¹ every 3 min and during the final 30 seconds of each 3 min stage, blood lactate was assessed using capillary blood samples (Lactate Plus, Nova Biomedical USA). Part 1 of the test terminated once both lactate threshold and turnpoint had been visually identified (defined as ≥ 0.4 mmol.L⁻¹ and ≥ 1.0 mmol.L⁻¹ above resting values respectively, 30). After a 5-min resting period, Part 2 of the test commenced at a velocity of 2 km.h⁻¹ below lactate turnpoint and the treadmill speed was increased by 1 km.h⁻¹ every minute until volitional fatigue or until completion of the 16 km.h⁻¹ stage, after which point the treadmill inclined by 1% every minute until volitional fatigue. $\dot{V}O_{2peak}$ was taken as the highest $\dot{V}O_2$ value obtained in any 10-sec period matching two of the following criteria: heart rate within 10 beats per min of age-predicted maximum, respiratory exchange ratio (RER) > 1.1 and plateau of oxygen consumption despite increased workload. To calculate $v\dot{V}O_{2peak}$, the final treadmill speed was used if the velocity was ≤ 16 km.h⁻¹ and where participants terminated the test during the inclined component at 16 km.h⁻¹, the following equation was used (30):

$$\text{Velocity at } \dot{V}O_{2peak} = (\dot{V}O_{2max} \times 60) / \text{Running economy}$$

$$Kph = (ml.kg^{-1}.min^{-1} \times 60) / ml.kg^{-1}.km^{-1}$$

$$\text{Running economy} = \dot{V}O_2 / (16 / 60)$$

$$ml.kg^{-1}.km^{-1} = ml.kg^{-1}.min^{-1} / (16 / 60)$$

Experimental Protocol:

Day 1: Participants arrived at the laboratory on the evening (17.00) of Day 1 having avoided alcohol and vigorous physical activity for the previous 24 h. Body mass was recorded and a heart rate (HR) monitor (Polar FT1, Finland) fitted. Participants then performed an intermittent running protocol on a motorised treadmill (HP, Cosmos) lasting ~90-120 min in an attempt to deplete muscle glycogen and thus allow for exercise-dietary standardisation prior to the outdoor training sessions. This exercise protocol has been used previously in our laboratory (31) and was chosen in an attempt to deplete muscle glycogen in both type I and type II muscle fibres. The activity pattern and total time to exhaustion were recorded, and water was consumed ad libitum throughout exercise. These parameters were repeated exactly during the second and third experimental trials. Within 30 minutes of completion of the depletion protocol, participants consumed 1.2 g.kg⁻¹ CHO in the form of sports drinks and bars (Lucozade, UK) and a 25 g whey protein solution (Upbeat Whey, UK). At 2 h after completion of the depletion protocol, participants also consumed a standardised meal containing 2 g.kg⁻¹ CHO, 40 g protein and 15 g fat.

Day 2: Subjects did not perform any structured training on Day 2 and also adhered to a standardised dietary intake of 6 g.kg⁻¹ CHO, 2 g.kg⁻¹ protein and 1 g.kg⁻¹ fat.

Day 3: After adhering to a further standardised dietary intake of 6 g.kg⁻¹ CHO, 2 g.kg⁻¹ protein and 1 g.kg⁻¹ fat on Day 3 (consumed during the period between 0700 h and

1500 h), subjects commenced one of the three training sessions at approximately 1600 h. Both the 8 x 800 m and 3 x 10 min track interval sessions were completed at an outdoor athletics track (Wavertree Athletics track, Liverpool, UK). The 10-mile road run was commenced on an outdoor course (designed by the first author) that commenced and finished at the Research Institute for Sport and Exercise Sciences at Liverpool John Moores University. Upon arrival at each respective trial location, a resting venous blood sample and muscle biopsy were obtained from the VL and lateral head of the G muscle. Biopsies taken from each muscle group were from opposite legs (i.e. right vastus lateralis and left gastrocnemius) and subsequent trials sampled the opposite leg to the previous trial. Muscle samples were immediately snap frozen in liquid nitrogen. Participants wore a GPS watch (Garmin Fore Runner 620) and heart rate (HR) monitor (Garmin) during exercise in all trials to verify the correct exercise intensity. During the 10-mile road run, the first author accompanied the participant (via cycling alongside) and the participant reported their rating of perceived exertion (RPE, 26) at the end of every mile. During the 8 x 800 m track run, participants reported RPE upon completion of each interval and capillary blood lactate was also sampled in the 30 seconds after completion of interval 2, 4, 6 and 8 (Lactate Plus, Nova Biomedical USA). During the 3 x 10 min track interval run, capillary blood lactate and RPE were also recorded at the end of each 10-minute interval. Upon completion of all three exercise trials, post-exercise venous blood sample and VL and G muscle biopsies (at 2 cm distal to the pre-exercise biopsy from the same leg) were also obtained.

Muscle biopsies: Muscle biopsies were obtained from the VL and lateral head of the G muscle within 5 minutes of commencing and completing each training session (Bard Monopty Disposable Core Biopsy Instrument 12 guage x 10 cm length, Bard Biopsy Systems, Tempe, AZ, USA). Samples were obtained under local anaesthesia (0.5%

235 marcaine) and immediately frozen in liquid nitrogen and stored at – 80°C for later
236 analysis.

237 ***Muscle glycogen concentration:*** Muscle glycogen concentration was determined
238 according to the acid hydrolysis method described by Van Loon et al. (32) with glucose
239 concentration quantified using a commercially available kit (GLUC-HK, Randox
240 Laboratories, Antrim, UK).

241 ***Blood analysis:*** Venous blood samples were collected in vacutainers containing K₂
242 EDTA, lithium heparin or serum separation tubes, and stored on ice until centrifugation
243 at 1500 g for 15 min at 4 °C. Plasma samples were aliquoted and stored at -80 °C until
244 analysis. Plasma glucose, lactate, non-esterified fatty acids (NEFA) and glycerol were
245 analysed using the Randox Daytona spectrophotometer with commercially available
246 kits (Randox, Ireland), as per the manufacturer's instructions.

247 ***Statistical analysis:*** Randomisation of training sessions was balanced for both males
248 and females by stratifying the randomisation by gender. The randomisation schedule
249 was generated according to a Williams square for a 3 by 3 cross-over study. The
250 planned sample size of 20 participants completing the study had 90% power to detect a
251 glycogen utilisation of 85 mmol/kg dry muscle (standard error = 34) for each of the
252 exercise protocol sessions (as based on similar running training sessions studied in our
253 laboratory (11, 13), at the two-sided 1.7% significance level (the effects of sex were
254 studied as an exploratory analysis). The study outcomes were analysed using a linear
255 mixed model for parameters that were considered Normally distributed, and non-
256 parametric methods (Wilcoxon signed ranks test), otherwise. In the linear model, the
257 dependent variable was the outcome of interest, and the independent variables included
258 main effects for participant, exercise protocol, study period, gender, muscle type and

baseline (where applicable). Interactions between gender and muscle type with exercise protocol were evaluated and were retained in the model if comparisons were to be made within subgroups, otherwise and if non-significant ($P>0.05$) these interaction terms were dropped from the model. Parameters associated with the anthropometric profile, training profile and physiological profile were compared between males and females using a t-test. P-values were not adjusted for multiplicity.

Results

Overview of training workloads

A comparison of workloads (i.e. exercise duration and distance ran) between each training session in males and females is displayed in Table 2. The time taken to complete the exercise protocols with set distances (i.e. the 10-mile and 8 x 800 m sessions) was significantly different between sexes such that males completed both training sessions faster than females ($P<0.001$). In males, the total exercise duration was different between training protocols such that 10 mile > 3 x 10 min > 8 x 800 ($P<0.01$ for all comparisons). Similarly, in female participants, the time required to complete the 10-mile road run was slower than both the 8 x 800 and 3 x 10 min session ($P<0.001$) though no difference was apparent between the track training sessions. In relation to the training protocol with set duration (i.e. the 3 x 10 min track training session), males completed more distance compared with females ($P<0.001$).

Physiological and metabolic responses to training

Changes in plasma metabolites during exercise are displayed in Figure 1. Plasma glucose did not significantly change during any of the exercise protocols ($P=0.6, 0.9$

and 0.8 for 10-mile, 8 x 800 m and 3x10 min, respectively). In contrast, exercise increased both NEFA ($P<0.01$ for all exercise protocols) and glycerol ($P<0.01$ for all exercise protocols) in all training protocols whereas exercise only increased plasma lactate ($P=0.002$) in the 8 x 800 m protocol. In relation to plasma NEFA, exercise-induced changes in NEFA were greater in the 10-mile session compared with both 8 x 800 m ($P=0.004$) and 3 x 10 min protocols ($P=0.003$) whilst the 3 x 10 min protocol was also significantly greater than the 8 x 800 m protocol ($P=0.006$). Finally, plasma glycerol responses were also significantly greater in both the 10-mile ($P=0.02$) and 8 x 800 m ($P=0.02$) when compared with the 3 x 10 min protocol. Whilst females displayed significantly greater increases in plasma glycerol than males in the 10-mile run ($P=0.01$), there was no difference in plasma metabolite responses between males and females in the remaining training protocols ($P>0.05$ for all comparisons).

Resting muscle glycogen concentration and glycogen utilisation during training

Muscle glycogen concentration before and after each training protocol is displayed in Figure 2, where statistical comparisons of training protocol, sex and muscle group on resting glycogen concentration are visually annotated. When comparing resting muscle glycogen concentration between exercise protocols, no significant differences were evident within each sex and muscle ($P>0.05$) with the exception of the G muscle in the female participants where pre-training glycogen concentration was lower in the 8 x 800 m protocol compared with both the 10-mile road run ($P=0.02$) and 3 x 10 min track run ($P=0.01$).

In relation to sex-specific differences in resting muscle glycogen concentration, females displayed reduced muscle glycogen concentration in the G muscle when compared with

males ($P<0.001$) though no such differences between sexes were apparent in the VL muscle ($P=0.40$). In relation to differences in resting glycogen concentration between muscles, the G muscle displayed higher glycogen concentration than the VL in males ($P<0.01$) though no such differences were evident in females ($P=0.78$) (Figure 1).

Total muscle glycogen utilisation during exercise (as calculated from pre-training minus post-training values) is presented in Table 3. Within the G muscle of male participants, there was a significant difference between training protocols such that 10-mile $>$ 8 x 800 m $>$ 3 x 10-min ($P<0.05$ for all comparisons). Similarly, glycogen utilisation within the VL muscle of male participants was greater in the 10-mile compared with both the 8 x 800 m and 3 x 10-min ($P<0.01$ for both comparisons) though no differences were apparent between the track running sessions ($P=0.64$). In contrast, total glycogen utilisation in the female participants was not statistically different between training protocols in both the G and VL muscles ($P>0.05$ for all comparisons). When comparing sex-specific responses, total glycogen utilisation was greater in males than females in both the VL ($P=0.02$) and G ($P=0.07$) muscle during the 10-mile road run only. With the exception of males during the 3 x 10 min protocol ($P=0.28$), greater absolute glycogen utilisation was observed in the G versus the VL muscle in both males and females and during all training protocols ($P<0.05$ for all comparisons) (Table 3).

Rates of muscle glycogen utilisation (as calculated by total glycogen utilisation divided by training duration) are presented in Table 4. In male participants within the G muscle, there was a significant difference between training protocols such that 8 x 800 m $>$ 3 x 10-min $>$ 10-mile road run ($P<0.05$ for all comparisons). Similarly, rates of glycogen utilisation within the VL muscle of male participants was greater in the 8 x 800 m compared with the 10-mile road run ($P=0.003$) though no differences were apparent

between the track running sessions. In female participants, rates of glycogen utilisation were greater in both the 8 x 800 m and 3 x 10-min within the G muscle compared with the 10-mile road run ($P < 0.01$ for both comparisons) though no differences were apparent between the track running sessions. In contrast, there was no difference in rates of glycogen utilisation with the VL muscle of female participants between training sessions ($P > 0.05$ for all comparisons). When comparing sex-specific responses, rate of glycogen utilisation was greater in males than females ($P < 0.01$) in the G muscle during the 8 x 800 m track run only. Finally, there was a significant main effect of muscle group in that higher rates of utilisation was typically observed in the G versus the VL muscle in both males and females and during all training protocols ($P < 0.01$).

Discussion

The aim of the present study was to quantify glycogen utilization of the vastus lateralis and gastrocnemius muscles of recreationally active male and female runners during three types of outdoor training sessions that are considered representative of runners' habitual workouts. Importantly, this is the first time that the effect of training protocol, sex and muscle sampled on net muscle glycogen utilisation has been simultaneously investigated within the same study. Our data demonstrate that 1) prolonged steady state running necessitates a higher absolute glycogen requirement than shorter but higher intensity track running sessions, 2) females display evidence of reduced resting muscle glycogen concentration and net muscle glycogen utilisation when compared with males and 3), net glycogen utilisation is higher in the gastrocnemius muscle compared with the vastus lateralis. Whilst the pattern of glycogen utilisation observed here is, of course, specific to the training status of the participants and the characteristics of the chosen

exercise protocols, our data may help to inform practical guidelines in relation to fuelling strategies to promote both training intensity and metabolic adaptations.

In an attempt to standardise resting muscle glycogen concentration between trials, all runners completed an initial bout of glycogen depleting exercise followed by 48 h of standardised dietary CHO intake equating to 6 g.kg⁻¹ per day. In this way, our experimental design allowed us to more accurately assess the effects of exercise protocol, sex and muscle group on net exercise-induced muscle glycogen utilisation.

Although we observed resting glycogen concentrations in the vastus lateralis muscle of males (i.e. 400-500 mmol.kg⁻¹ dw) that is consistent with the fitness level (i.e. 50 ml.kg⁻¹ min⁻¹) and dietary CHO intake (i.e. 2 days of 6 g.kg⁻¹) reported in a recent meta-analysis (23), comparison of resting glycogen concentrations between muscles and sex also revealed a number of interesting findings. Firstly, we observed that resting glycogen concentration in the gastrocnemius muscle of males was higher than that of the vastus lateralis. Secondly, we also observed that females displayed reduced resting glycogen concentration in the gastrocnemius muscle compared with males. Whilst it is currently difficult to offer definitive mechanisms underpinning such findings, it is possible that the combination of glycogen depleting exercise coupled with the lower absolute CHO intake in females (6 g/kg body mass equating to 360 g CHO) compared with males (6 g/kg body mass equating to 460 g CHO) may have contributed, in part, to these results. Indeed, given that the magnitude of post-exercise muscle glycogen re-synthesis is well known to be dependent on the extent of prior glycogen depletion (33) and that the gastrocnemius muscle was likely depleted to a greater extent than the vastus lateralis (as reported by Areta and Hopkins, 23, and later verified in Table 2), it is suggested that the elevated resting glycogen concentration in the gastrocnemius muscle in male participants may possibly be a reflection of greater absolute utilisation during

the depletion and subsequent re-synthesis in response to a given exercise stimulus and dietary CHO intake.

In relation to sex-specific differences, a reduced capacity of females to store glycogen in the vastus lateralis muscle (as also assessed in the follicular phase) compared with males has also been reported previously by Tarnopolsky et al. (21), as evidenced in response to a 3 day CHO loading protocol consisting of cycling based exercise and elevated dietary CHO intake (increased CHO intake from 55 to 75% of habitual energy intake). Using this approach, the authors observed an approximate 150 mmol.kg⁻¹ dw difference in glycogen storage between males and females. The authors suggested that such differences may be due to the combination of greater prior glycogen depletion in males compared with females in addition to a higher absolute CHO intake in males (8 g/kg body mass equating to 610 g CHO) compared with females (6 g/kg body mass equating to 370 g CHO). The same group later demonstrated that when females complete a 4 day CHO loading protocol where a higher relative (9 g/kg body mass) and absolute CHO intake is consumed (540 g CHO), no differences in glycogen concentration is apparent when compared with males who consume a comparable absolute dose (600 g CHO equating to 8 g/kg body mass) (25,26). When considered this way, it is possible that the shorter duration of dietary standardisation (i.e. 2 days) utilised here coupled with the lower absolute CHO intake consumed by females may have contributed to the present findings. Whilst the pre-exercise glycogen availability achieved here was sufficient to fuel the workloads of the present training protocols, our data perhaps add further evidence to the suggestion that females require greater relative CHO intakes than males in order to achieve comparable absolute CHO intakes and subsequent CHO loading responses (likely to be especially relevant when the training session is more prolonged in nature).

In relation to the glycogen requirement of specific training sessions, we observed that the net glycogen utilisation in males was greatest in the 10-mile road run ($\approx 70\% \dot{V}O_{2\text{peak}}$) when compared with both the 8 x 800 m ($100\% \dot{V}O_{2\text{peak}}$) and 3 x 10-min track runs ($\approx 80\% \dot{V}O_{2\text{peak}}$), a pattern of utilisation that was evident in both the gastrocnemius and vastus lateralis muscles. Additionally, net glycogen utilisation in gastrocnemius muscle was also greater in the 8 x 800 m training session when compared with the 3 x 10-min session, though such a difference between the track sessions was not evident in the vastus lateralis muscle. When considering such data in combination with the greater net (and rates of) glycogen utilisation observed in the gastrocnemius muscle compared with the vastus lateralis (see Table 3 and 4), our data extend the classical findings of Costill et al. (20) highlighting that the gastrocnemius muscle is a more suitable muscle (i.e. as reflective of greater muscle fibre recruitment) for which to study glycogen metabolism during running given its sensitivity to detect changes of physiological significance.

The absolute net glycogen utilisation induced by a specific training session is, of course, a product of exercise duration and exercise intensity. In accordance with post-exercise circulating lactate concentrations (see Figure 1), it is noteworthy that the highest rates of glycogen utilisation was also observed in the gastrocnemius muscle during the 8 x 800 m training session. Similarly, the highest rate of glycogen utilisation in the vastus lateralis was also observed during the 8 x 800 m session. In contrast to the male participants, however, no differences in net glycogen use between training protocols were evident in the female participants, despite differences in rates of glycogen utilisation between certain training sessions. Whilst such data may be related, in part, to the fact that relative training intensity in the females did not differ between training protocols (i.e. 80-100% $\dot{V}O_{2\text{max}}$) to the same extent for male participants (i.e. 70-100%

VO_{2max}), our data clearly highlight how the interplay between muscle fibre recruitment, relative exercise intensity and training duration can all modulate the absolute muscle glycogen requirement associated with a specific exercise protocol.

The methodological difficulties of isolating the effects of sex on substrate utilisation during exercise have been well documented (22), arising from factors relating to matching of participant characteristics, relative exercise intensity, exercise duration and of course, overall absolute work done. To this end, we deliberately chose to study the effects of sex on glycogen utilisation during three real world training sessions comprising training at identical relative exercise intensities and distance ran (i.e. both the 10-mile road run and 8 x 800 m track session) as well as a session that was matched for relative training intensity but also in training duration (i.e. 3 x 10 minute track session). We observed no statistical differences between absolute or rates of glycogen utilisation between males and females in either the gastrocnemius or vastus lateralis muscles during the track based training sessions (see Table 3 and 4). Such a finding may be related to the fact that these sessions were completed at relative exercise intensities that are already sufficient to activate regulatory enzymes of glycogenolysis and glycolysis whilst also suppressing NEFA uptake and oxidation by the mitochondria.

In contrast, we observed sex specific responses in absolute muscle glycogen utilisation in both the vastus lateralis (P=0.02) and gastrocnemius muscle (P=0.07) during the 10-mile road run. Importantly, this run was completed at a running velocity corresponding to lactate threshold (as opposed to a % of VO_{2peak}) given that matching relative exercise intensity according to threshold is considered a more accurate method to assess CHO metabolism within (34,35) and between sexes (36,37). It is, of course, possible that the differences in net glycogen utilisation between sexes may be due to the fact that females presented with lower resting glycogen concentration as well actual differences in time

taken to complete the 10-mile distance, especially when considering that rates of glycogen utilisation in both muscles were not statistically different between males and females (though approximate differences of $1 \text{ mmol.kg}^{-1} \text{ min}^{-1}$ could be considered of physiological relevance during prolonged exercise). Nonetheless, data do appear consistent with previous observations that females exhibit a lower respiratory exchange ratio during exercise, thus indicative of less reliance on whole body CHO metabolism to support substrate metabolism during sub-maximal steady-state exercise (27,28). Whilst such differences have been demonstrated to be reflective of differences in liver glycogenolysis (27, 36), it is noteworthy that our data also appear consistent with the observation that females utilise less muscle glycogen during running (21) but not cycling (28). Indeed, the former authors observed that absolute glycogen utilisation in the vastus lateralis was reduced by approximately 25% in females compared with males when both groups completed a set running distance of 15.5 km at a relative exercise intensity corresponding to 65% $\text{VO}_{2\text{max}}$, a similar magnitude of difference and running distance as to that studied here. Such observations suggest that running may be a more suitable exercise modality for which to study sex differences in substrate metabolism during exercise, especially when exercise intensity is sub-maximal and matched according to lactate threshold (37). Nonetheless, we also acknowledge the requirement to study both fibre type specific differences in glycogen and intramuscular triglyceride metabolism as well as the kinetics of lipid metabolism, as opposed to the limitations of whole muscle homogenate and static measures of post-exercise NEFA and glycerol concentrations utilised here. Additionally, a comparison of males and females at varying stages throughout the menstrual cycle (completing the types of exercise protocols studied here) is also a future research recommendation.

When taken together, our data illustrate how the complex interplay between muscle group, specifics of training protocol and sex can all modulate the net glycogen requirement associated with a given exercise stress. In addition to informing future research design methodology, our data may be of practical significance in helping to formulate CHO requirements in relation to specific types of training sessions. Indeed, the resting glycogen concentrations achieved by the 2-day dietary CHO intake of 6 g/kg body mass were sufficient to fuel the workloads of the training protocols studied here. Additionally, whilst we observed small differences in substrate storage and metabolism between sexes, it is unlikely that such differences would manifest as sex-specific practical recommendations for the types of training intensities and duration studied here. Finally, it is noteworthy that all subjects were able to sustain the required training intensity during the 10-mile road run in the absence of CHO feeding during exercise. Such data may also be of practical relevance when considering that CHO feeding during exercise may actually attenuate training-induced oxidative adaptations of human skeletal muscle (13), though it is acknowledged that such studies have not yet been performed in females.

In summary, we conclude that 1) prolonged steady state running necessitates a higher absolute glycogen requirement than shorter but higher intensity track running sessions, 2) females display evidence of reduced resting muscle glycogen concentration and absolute muscle glycogen utilisation when compared with males and 3), both absolute and rates of glycogen utilisation are higher in the gastrocnemius muscle compared with the vastus lateralis. Whilst such observations are specific to the training status of the participants studied here, our data may provide a platform to help better inform CHO periodization strategies for runners and will hopefully stimulate further research.

Disclosure

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Author Contributions

SGI, AH, SOS and JPM conception and design of research; SGI, EJ, GM, MC, JS, NC, SOS and JPM performed experiments; SGI, DT, SOS and JPM analyzed data; SGI, DT, SOS and JPM interpreted results of experiments; SGI and JPM prepared the figures; SGI, DT, SOS and JPM drafted the manuscript; SGI, EJ, GM, MC, JS, NC, IL, DT, AH, SOS and JPM edited and revised the manuscript; SGI, EJ, GM, MC, JS, NC, IL, DT, AH, SOS and JPM approved the final version of manuscript.

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Table 1: Anthropometric profile, training history and physiological profile of male and female participants. * denotes significant difference between males and females, $P < 0.05$. Values presented are means \pm SD.

Table 2: Summary of training protocols. * denotes significant difference between males and females; Groups with different letters (males) and numbers (females) denotes significant difference, $P < 0.05$. Values presented are adjusted means (SE) from mixed model with terms for training protocol, gender, training protocol by gender interaction, period and subject. For total distance, comparisons between males and females are made for the 3 x 10 min protocol only (t-test).

Table 3: Total muscle glycogen utilisation in gastrocnemius and vastus lateralis muscles groups during each training protocol. * denotes significant difference between males and females, $P < 0.05$. ** denotes higher utilisation in the gastrocnemius muscle, $P < 0.05$. Groups with different letters (males) and numbers (females) denotes significant difference, $P < 0.05$. Values presented are adjusted means (SE) from mixed model with terms for training protocol, gender, muscle, training protocol by gender by muscle interaction, period, subject and baseline glycogen.

Table 4: Rates of muscle glycogen utilisation in gastrocnemius and vastus lateralis muscles groups during each training protocol. * denotes significant difference between males and females, $P < 0.05$. ** denotes higher utilisation in the gastrocnemius muscle, $P < 0.05$. Groups with different letters (males) and numbers (females) denotes significant difference, $P < 0.05$. Values presented are adjusted means (SE) from mixed model with terms for training protocol, gender, muscle, training protocol by gender by muscle interaction, period, subject and baseline glycogen.

Figure 1: Plasma (A) lactate, (B) glucose, (C) NEFA and (D) glycerol pre- and post-exercise in the 10 mile road run, 8 x 800 m and 3 x 10 min track runs (panels left to right respectively). * denotes significant difference effect of exercise, $P < 0.05$. a denotes significant difference from 10 mile and 3 x 10 min training sessions, $P < 0.05$. b denotes significant difference from the 3 x 10 min training session, $P < 0.05$.

Figure 2: Muscle glycogen in the G and VL muscles pre- and post- the 10 mile road run (A and B), the 8 x 800 m track run (C and D) and the 3 x 10 min track run (E and F). * denotes significantly lower resting concentration in the G muscle of females compared with males, $P < 0.05$. a denotes significantly lower resting concentration within the G muscle of females when compared with the 10 mile and 3 x 10 min trials, $P < 0.05$. b denotes significantly higher resting concentration in the G muscle of males compared with the VL muscle, $P < 0.05$. For statistical comparisons of training protocol, sex and muscles on glycogen utilisation, please see Table 2.

TABLE 1

	Males (n=11)	Females (n=10)
Anthropometric Profile		
Age (years)	25.3 ± 3.4	24.3 ± 3.4
Body Mass (kg)	76.2 ± 7.6	61.5 ± 7.1 *
Height (cm)	178.5 ± 5.4	167.1 ± 8.0 *
Fat Free Mass (kg)	59.0 ± 6.1	40.0 ± 5.5 *
% Body Fat	14.4 ± 3.7	27.5 ± 2.8 *
Training Profile		
Weekly distance (km)	34.9 ± 21.2	21.1 ± 11.4
Weekly duration (hours)	4.6 ± 2.0	2.9 ± 0.8 *
Physiological Profile		
VO _{2peak} (L/min)	4.2 ± 0.4	2.6 ± 0.4 *
VO _{2peak} (mL.kg ⁻¹ .min ⁻¹)	53.9 ± 4.7	42.6 ± 4.0 *
VO _{2peak} (mL.kg ⁻¹ FFM.min ⁻¹)	69.7 ± 6.1	65.6 ± 5.8
vVO _{2peak} (km.h ⁻¹)	16.5 ± 0.7	13.9 ± 1.2 *
Lactate Threshold (% VO _{2peak})	68.6 ± 6.3	77.3 ± 5.5 *
Lactate Threshold (km.h ⁻¹)	12.5 ± 0.7	10.2 ± 0.9 *
Lactate Turnpoint (% VO _{2peak})	76.4 ± 6.1	81.7 ± 7.7
Lactate Turnpoint (km.h ⁻¹)	13.6 ± 0.7	11.2 ± 0.9 *

TABLE 2

Protocol Description	10-mile	8 x 800 m	3 x 10 min
	10-mile (16.1 km) road run at velocity at lactate threshold	8 x 800 m on running track at $v\text{VO}_{2\text{ peak}}$ with 2-min recovery period between each repetition	3 x 10-min intervals on running track at velocity at lactate turn-point with 2-min recovery period between each repetition
Total Duration (min)			
Males	77.3 (1.5) ^{*a}	23.4 (1.4) ^{*b}	30 ^c
Females	96.4 (1.7) ¹	28.3 (1.8) ²	30 ²
Total Distance (km)			
Males	16.1	6.4	6.8 (0.4) *
Females	16.1	6.4	5.6 (0.6)

TABLE 3

	10-mile	8 x 800 m	3 x 10 min
<i>Gastrocnemius</i> ** (mmol.kg ⁻¹ dw)			
Male	354 (24.7) ^a	288 (24.0) ^b	190 (26.1) ^c
Female	285 (27.2) ¹	230 (30.2) ¹	254 (24.7) ¹
<i>Vastus Lateralis</i> (mmol.kg ⁻¹ dw)			
Male	265 (24.1) * ^a	166 (22.8) ^b	151 (24.1) ^b
Female	179 (27.2) ¹	142 (28.9) ¹	139 (24.3) ¹

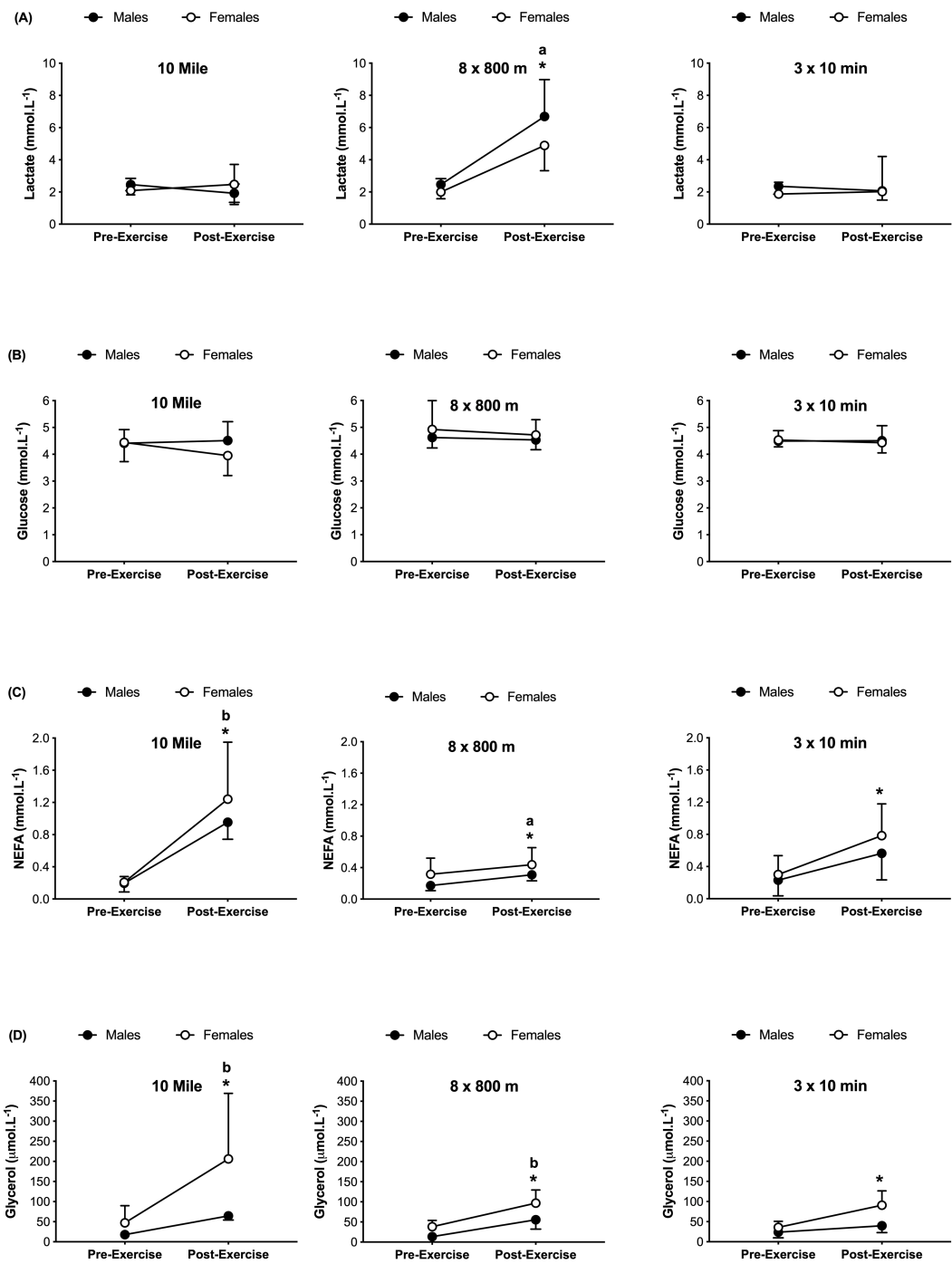
TABLE 4

	10-mile	8 x 800 m	3 x 10 min
<i>Gastrocnemius</i>** (mmol.kg ⁻¹ .min ⁻¹)			
Male	4.2 (0.9) ^a	12.7 (0.9) ^{*b}	7.4 (0.9) ^c
Female	2.8 (1.0) ¹	7.5 (1.1) ²	8.6 (0.9) ²
<i>Vastus Lateralis</i> (mmol.kg ⁻¹ .min ⁻¹)			
Male	3.2 (0.9) ^a	7.0 (0.9) ^b	4.9 (0.9) ^{a,b}
Female	2.5 (1.0) ¹	5.1 (1.1) ¹	4.5 (0.9) ¹

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