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

64

Introduction

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

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

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

In addition to the specifics of the exercise protocol, differences in muscle group examined (12,20) and sex-specific alterations in substrate metabolism (21) may also affect the absolute glycogen utilisation associated with specific training sessions. To our knowledge, however, such comparisons of muscle group, sex, and training intensity have not yet been simultaneously examined within the same study. The paucity of data from female participants was recognized by Devries et al. (2016) (22) though is much 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) competed 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%

marcaine) and immediately frozen in liquid nitrogen and stored at – 80ºC for later analysis.

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

Blood analysis: Venous blood samples were collected in vacutainers containing K_2 EDTA, lithium heparin or serum separation tubes, and stored on ice until centrifugation at 1500 g for 15 min at 4℃. Plasma samples were aliquoted and stored at -80℃ until analysis. Plasma glucose, lactate, non-esterified fatty acids (NEFA) and glycerol were analysed using the Randox Daytona spectrophotometer with commercially available kits (Randox, Ireland), as per the manufacturer's instructions.

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

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

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

325 Rates of muscle glycogen utilisation (as calculated by total glycogen utilisation divided
326 by training duration) are presented in Table 4. In male participants within the G muscle,
327 there was a significant difference between training protocols such that 8 x 800 m > 3 x
328 10-min > 10-mile road run (P<0.05 for all comparisons). Similarly, rates of glycogen
329 utilisation within the VL muscle of male participants was greater in the 8 x 800 m
330 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

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

357 In an attempt to standardise resting muscle glycogen concentration between trials, all
358 runners completed an initial bout of glycogen depleting exercise followed by 48 h of
359 standardised dietary CHO intake equating to 6 $g.kg^{-1}$ per day. In this way, our
360 experimental design allowed us to more accurately assess the effects of exercise
361 protocol, sex and muscle group on net exercise-induced muscle glycogen utilisation.
362 Although we observed resting glycogen concentrations in the vastus lateralis muscle of
363 males (i.e. 400-500 $mmol.kg^{-1}$ dw) that is consistent with the fitness level (i.e. 50 $ml.kg^{-1}$
364 min^{-1}) and dietary CHO intake (i.e. 2 days of 6 $g.kg^{-1}$) reported in a recent meta-
365 analysis (23), comparison of resting glycogen concentrations between muscles and sex
366 also revealed a number of interesting findings. Firstly, we observed that resting
367 glycogen concentration in the gastrocnemius muscle of males was higher than that of
368 the vastus lateralis. Secondly, we also observed that females displayed reduced resting
369 glycogen concentration in the gastrocnemius muscle compared with males. Whilst it
370 is currently difficult to offer definitive mechanisms underpinning such findings, it is
371 possible that the combination of glycogen depleting exercise coupled with the lower
372 absolute CHO intake in females (6 g/kg body mass equating to 360 g CHO) compared
373 with males (6 g/kg body mass equating to 460 g CHO) may have contributed, in part,
374 to these results. Indeed, given that the magnitude of post-exercise muscle glycogen re-
375 synthesis is well known to be dependent on the extent of prior glycogen depletion (33)
376 and that the gastrocnemius muscle was likely depleted to a greater extent than the vastus
377 lateralis (as reported by Areta and Hopkins, 23, and later verified in Table 2), it is
378 suggested that the elevated resting glycogen concentration in the gastrocnemius muscle
379 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).

405 In relation to the glycogen requirement of specific training sessions, we observed that
406 the net glycogen utilisation in males was greatest in the 10-mile road run (≈ 70%
407 $\dot{V}O_{2peak}$) when compared with both the 8 x 800 m (100% $\dot{V}O_{2peak}$) and 3 x 10-min track
408 runs (≈ 80% $\dot{V}O_{2peak}$), a pattern of utilisation that was evident in both the gastrocnemius
409 and vastus lateralis muscles. Additionally, net glycogen utilisation in gastrocnemius
410 muscle was also greater in the 8 x 800 m training session when compared with the 3 x
411 10-min session, though such a difference between the track sessions was not evident in
412 the vastus lateralis muscle. When considering such data in combination with the
413 greater net (and rates of) glycogen utilisation observed in the gastrocnemius muscle
414 compared with the vastus lateralis (see Table 3 and 4), our data extend the classical
415 findings of Costill et al. (20) highlighting that the gastrocnemius muscle is a more
416 suitable muscle (i.e. as reflective of greater muscle fibre recruitment) for which to study
417 glycogen metabolism during running given its sensitivity to detect changes of
418 physiological significance.

419 The absolute net glycogen utilisation induced by a specific training session is, of course,
420 a product of exercise duration and exercise intensity. In accordance with post-exercise
421 circulating lactate concentrations (see Figure 1), it is noteworthy that the highest rates
422 of glycogen utilisation was also observed in the gastrocnemius muscle during the 8 x
423 800 m training session. Similarly, the highest rate of glycogen utilisation in the vastus
424 lateralis was also observed during the 8 x 800 m session. In contrast to the male
425 participants, however, no differences in net glycogen use between training protocols
426 were evident in the female participants, despite differences in rates of glycogen
427 utilisation between certain training sessions. Whilst such data may be related, in part,
428 to the fact that relative training intensity in the females did not differ between training
429 protocols (i.e. 80-100% VO_{2max}) to the same extent for male participants (i.e. 70-100%

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

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

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

455 taken to complete the 10-mile distance, especially when considering that rates of
456 glycogen utilisation in both muscles were not statistically different between males and
457 females (though approximate differences of 1 mmol.kg^{-1} min^{-1} could be considered of
458 physiological relevance during prolonged exercise). Nonetheless, data do appear
459 consistent with previous observations that females exhibit a lower respiratory exchange
460 ratio during exercise, thus indicative of less reliance on whole body CHO metabolism
461 to support substrate metabolism during sub-maximal steady-state exercise (27,28).
462 Whilst such differences have been demonstrated to be reflective of differences in liver
463 glycogenolysis (27, 36), it is noteworthy that our data also appear consistent with the
464 observation that females utilise less muscle glycogen during running (21) but not
465 cycling (28). Indeed, the former authors observed that absolute glycogen utilisation in
466 the vastus lateralis was reduced by approximately 25% in females compared with males
467 when both groups completed a set running distance of 15.5 km at a relative exercise
468 intensity corresponding to 65% VO_{2max}, a similar magnitude of difference and running
469 distance as to that studied here. Such observations suggest that running may be a more
470 suitable exercise modality for which to study sex differences in substrate metabolism
471 during exercise, especially when exercise intensity is sub-maximal and matched
472 according to lactate threshold (37). Nonetheless, we also acknowledge the requirement
473 to study both fibre type specific differences in glycogen and intramuscular triglyceride
474 metabolism as well as the kinetics of lipid metabolism, as opposed to the limitations of
475 whole muscle homegenate and static measures of post-exercise NEFA and glycerol
476 concentrations utilised here. Additionally, a comparison of males and females at
477 varying stages throughout the menstrual cycle (completing the types of exercise
478 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 ± 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 *

744 TABLE 2

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	10-mile	8 x 800 m	3 x 10 min
Protocol Description	10-mile (16.1 km) road run at velocity at lactate threshold	8 x 800 m on running track at $vVO_{2\ peak}$ 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)

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TABLE 3

	10-mile	8 x 800 m	3 x 10 min
Gastrocnemius ** (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]
Vastus Lateralis (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
Gastrocnemius (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]
Vastus Lateralis (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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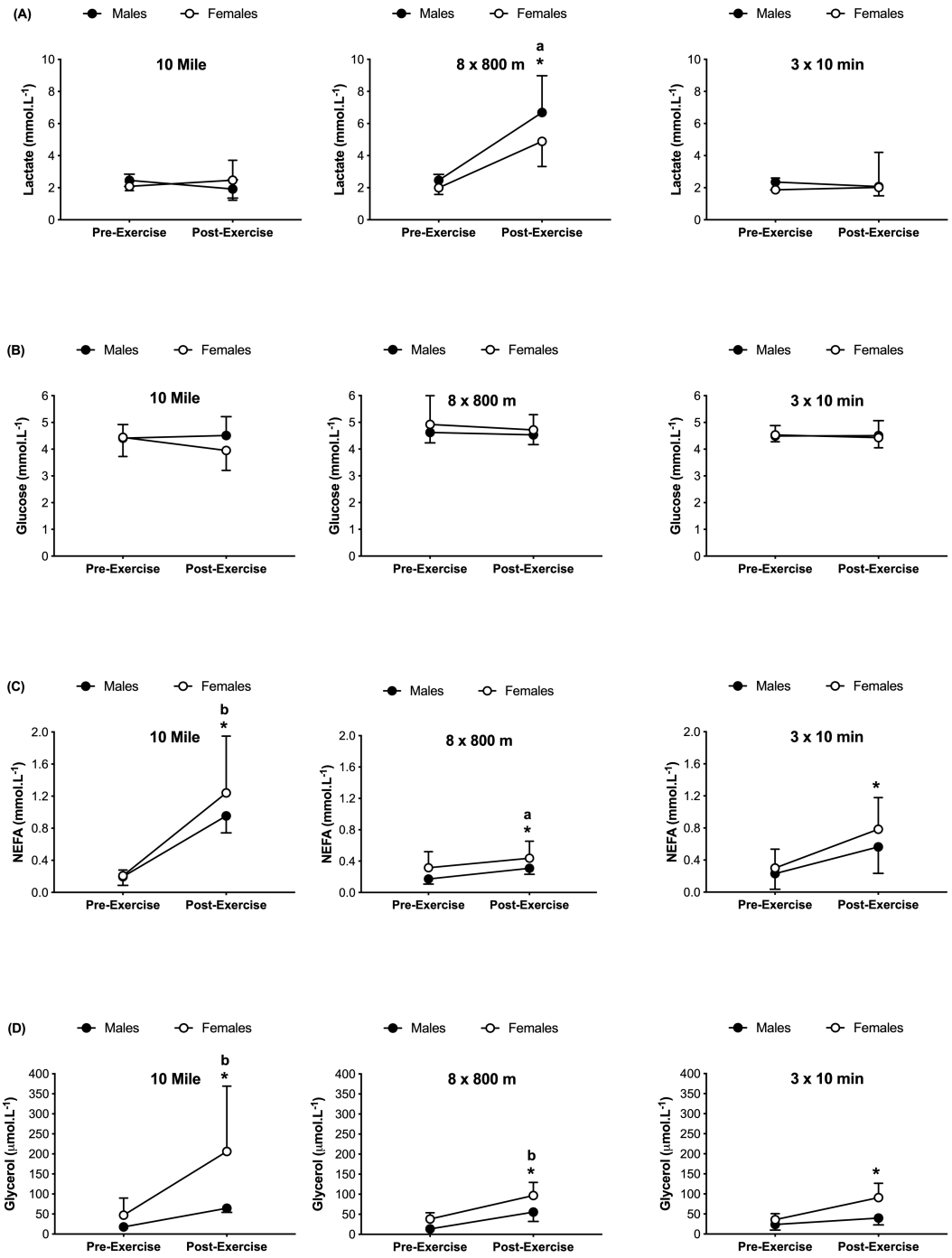

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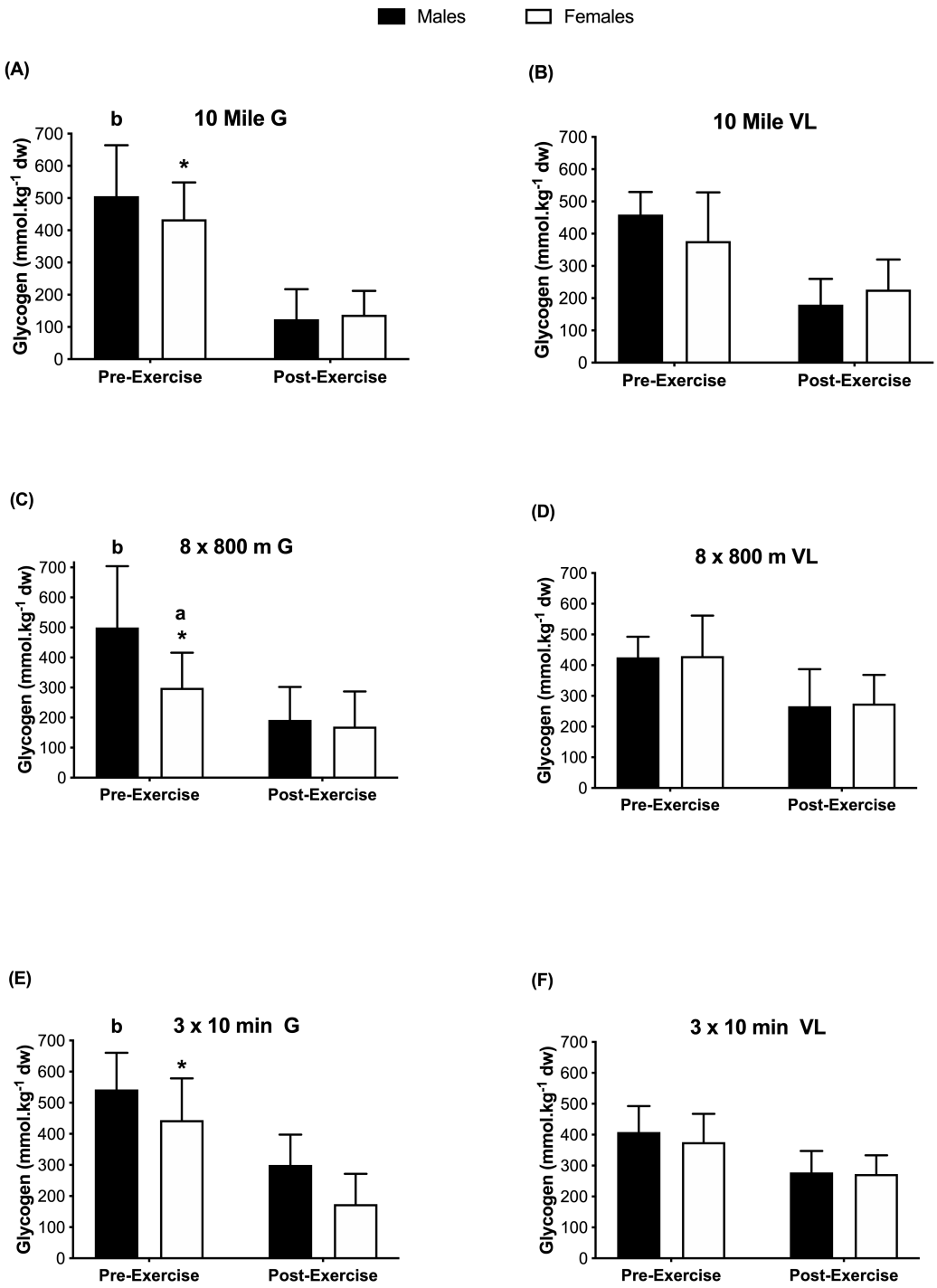
Males
Females
(A)
10 Mile G
b
*
Glycogen (mmol.kg-1 dw)
Pre-Exercise
Post-Exercise
(B)
10 Mile VL
(C)
8 x 800 m G
b
a
*
(D)
8 x 800 m VL
(E)
3 x 10 min G
b
*
(F)
3 x 10 min VL

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