Impact of Muscle Glycogen Availability on the Capacity for Repeated Exercise in Man

ABDULLAH F. ALGHANNAM1, DAWID JEDRZEJEWSKI1, MARK G. TWEDDLE1, HANNAH GRIBBLE1, JAMES BILZON1, DYLAN THOMPSON1, KOSTAS TSINTZAS2, and JAMES A. BETTS1

1Human Physiology Research Group, Department for Health, University of Bath, Bath, UNITED KINGDOM; and 2School of Life Sciences, Queen’s Medical Center, Nottingham, UNITED KINGDOM

ABSTRACT

ALGHANNAM, A. F., D. JEDRZEJEWSKI, M. G. TWEDDLE, H. GRIBBLE, J. BILZON, D. THOMPSON, K. TSINTZAS, and J. A. BETTS. Impact of Muscle Glycogen Availability on the Capacity for Repeated Exercise in Man. Med. Sci. Sports Exerc., Vol. 48, No. 1, pp. 123–131, 2016. Purpose: This study aims to examine whether muscle glycogen availability is associated with fatigue in a repeated exercise bout following short-term recovery. Methods: Ten endurance-trained individuals underwent two trials in a repeated-measures experimental design, each involving an initial run to exhaustion at 70% of VO2max (Run 1) followed by a 4-h recovery and a subsequent run to exhaustion at 70% of VO2max (Run 2). A low-carbohydrate (L-CHO; 0.3 g kg body mass−1·h−1) or high-carbohydrate (H-CHO; 1.2 g kg body mass−1·h−1) beverage was ingested at 30-min intervals during recovery. Muscle biopsies were taken upon cessation of Run 1, after recovery, and at exhaustion during Run 2 in L-CHO (F2). In H-CHO, muscle biopsies were obtained after recovery, at the time point coincident with fatigue in L-CHO (F2), and at the point of fatigue during the subsequent exercise bout (F3). Results: Run 2 was more prolonged for participants on H-CHO (80 ± 16 min) than for participants on L-CHO (48 ± 11 min; P < 0.001). Muscle glycogen concentrations were higher at the end of recovery for participants on H-CHO (269 ± 84 mmol·kg dry mass−1) than for participants on L-CHO (157 ± 37 mmol·kg dry mass−1; P = 0.001). The rate of muscle glycogen degradation during Run 2 was higher with H-CHO (3.1 ± 1.5 mmol·kg dry mass−1·min−1) than with L-CHO (1.6 ± 1.3 mmol·kg dry mass−1·min−1; P = 0.05). The concentration of muscle glycogen was higher with H-CHO than with L-CHO at F2 (123 ± 28 mmol·kg dry mass−1; P < 0.01), but no differences were observed between treatments at the respective points of exhaustion (78 ± 22 mmol·kg dry mass−1·min−1 for H-CHO vs 72 ± 21 mmol·kg dry mass−1·min−1 for L-CHO). Conclusion: Increasing carbohydrate intake during short-term recovery accelerates glycogen repletion in previously exercised muscles and thus improves the capacity for repeated exercise. The availability of skeletal muscle glycogen is therefore an important factor in the restoration of endurance capacity because fatigue during repeated exercise is associated with a critically low absolute muscle glycogen concentration. Key Words: NUTRITION, METABOLISM, PERFORMANCE, SUCROSE

Endurance capacity during an initial prolonged exercise bout is primarily reliant on pre-exercise glycogen availability, such that muscle glycogen content exhibits a direct positive correlation with exercise time to exhaustion (6,35,36). Similarly, muscle glycogen repletion can impact the time required to recover functional capacity, with carbohydrate intake accelerating glycogen resynthesis and restoration of exercise capacity relative to no intake of carbohydrate (16,17,23). Furthermore, carbohydrate ingestion rate exhibits a dose-dependent relationship with the rate of muscle glycogen resynthesis up to an ingestion threshold of ~1.2 g kg body mass−1·h−1 (8). It is therefore logical to postulate that increasing carbohydrate intake might also exhibit a dose-dependent relationship with the restoration of exercise capacity following short-term recovery. However, while some information is available pertaining to muscle glycogen metabolism during a subsequent exercise bout (4,9,34), it remains merely an assumption that muscle glycogen availability is an important determinant of fatigue during a second bout of exercise following short-term recovery.

Few studies have examined the relationship between carbohydrate ingestion rate during recovery and restoration of exercise capacity, with most reporting no consistent pattern (7,15,41) and with only one reporting a dose-dependent relationship (7). Notwithstanding that the aforementioned studies did not provide any glycogen data, evidence indicates that glycogen resynthesis (in particular liver glycogen) is an important determinant of endurance capacity following short-term recovery (10). This is understandable given that liver glycogen content is preferentially resynthesized over muscle glycogen when modest amounts of carbohydrate (~0.3 g kg body mass−1·h−1) are ingested following an initial exhaustive exercise bout (10). Conversely, the capacity for repeated exercise has also been...
dissociated from skeletal muscle glycogen availability in other studies (4,7,9). It is therefore possible that fatigue during repeated exercise may manifest differently from an initial prolonged exercise bout, and the availability of muscle glycogen may not be the primary cause of fatigue during subsequent exercise under all conditions. Accordingly, there is an outstanding need for improved understanding of the relative importance of muscle glycogen availability in offsetting fatigue during a repeated exercise bout, as opposed to an initial bout, with implications for optimal carbohydrate feeding strategy during recovery to maximize not only glycogen resynthesis but also restoration of exercise capacity.

To this end, the current study nutritionally manipulated carbohydrate availability during short-term recovery to examine metabolic and ergogenic outcomes during subsequent exercise. Specifically, we sought to examine whether muscle glycogen availability is associated with fatigue in a repeated exercise bout following short-term recovery. Comparisons were therefore made between a low-carbohydrate (L-CHO) supplement sufficient only to restore hepatic glycogen with minimal rates of muscle glycogen resynthesis (10) and a high-carbohydrate (H-CHO) supplement designed to elicit high rates of muscle glycogen resynthesis (37). We hypothesized that the extended run time to fatigue expected with increasing carbohydrate intake is caused by a proportional acceleration of muscle glycogen resynthesis during recovery and thus greater glycogen availability during repeated exercise.

MATERIALS AND METHODS

Participants. Nine healthy recreationally active men and one woman participated in the study. The characteristics of the sample were as follows (mean ± SD): age, 21 ± 1 yr; body mass, 72.5 ± 8.2 kg; height, 180 ± 9 cm; VO$_{2\text{max}}$, 61 ± 6 mL·kg$^{-1}$·min$^{-1}$; weekly exercise duration, 5 ± 3 h. The participants had been informed of the possible risks and discomforts involved before they gave their voluntary consent to take part in the study. The current study has been approved by the local National Health Services Research Ethics Committee (Ref. 09/H0101/82; controlled clinical trial number ISRCTN87937960).

Preliminary measurements. Participants undertook preliminary testing on two separate occasions. The first preliminary visit included the determination of each participant’s submaximal oxygen uptake (VO$_2$) and maximal oxygen uptake (VO$_{2\text{max}}$) (31) on a motorized treadmill (Ergo ELG70; Woodway, Weil am Rhein, Germany). The data acquired from these tests were employed to calculate the treadmill speeds that elicited 60% and 70% of VO$_{2\text{max}}$. The second visit (familiarization trial) was completed at least 2 wk before the main trials and required each participant to undergo the exercise protocol used in the main trials (described later) without any tissue or venous blood collection, and participants only ingested water to nutrient provision at similar intervals during the main trials (Fig. 1). This trial aimed to accustom the participants to the experimental procedures and apparatus, in addition to fully familiarizing them with running to the point of volitional exhaustion and thereby diminishing any learning or trial-order effects (Run 1 and Run 2 times to exhaustion were 103 ± 17 and 36 ± 9 min, respectively). Expired gas samples were collected during this visit to confirm the estimated relative speeds that corresponded to the required intensity during the main trials, with any adjustments applied accordingly.

Experimental design. Each participant performed two main trials in a repeated-measures experimental design interspersed by an interval of ≥2 wk. A weighed dietary record was completed 48 h before the familiarization trial and before the commencement of the main trials (2638 ± 708 kcal·d$^{-1}$; 55% ± 5% carbohydrate, 17% ± 3% fat, and 28% ± 4% protein). Participants were provided a standardized meal (760 kcal; 57% carbohydrate, 24% protein, and 19% fat) in the evening (12 ± 1 h) before the familiarization trial and before each main trial. Participants were also requested to abstain from alcohol consumption and to refrain from strenuous physical activity for 48 h before the trial, with any light exercise recorded and matched during standardization of lifestyle for the ensuing trials.

The main trials required participants to run to the point of volitional exhaustion (Run 1) at 70% of VO$_{2\text{max}}$; this was followed by a 4-h recovery period, wherein participants ingested a L-CHO or H-CHO supplement. Following recovery, a second run to exhaustion (Run 2) at the same exercise intensity (i.e., 70% of VO$_{2\text{max}}$) was undertaken by each participant to assess restoration of exercise capacity. As has been successfully applied in previous studies that contrasted relative and absolute fatigue points to understand fatigue mechanisms in relation to running (36) and cycling (12), trial order required L-CHO to be completed first. A previous study (7) has reported that restoration of exercise capacity during short-term recovery can be dose-dependent (moderate-carbohydrate to H-CHO vs H-CHO intake). Differences in exercise time to

FIGURE 1—A schematic representation of the study protocol. +Body mass assessment; #fluid provision; ﬁ expired gas and blood sample; (V) muscle biopsy during L-CHO treatment; (Y) muscle biopsy during H-CHO treatment; (dashed columns) warm-up; (black column) run time to exhaustion in the L-CHO trial; (gray column) extended run time to exhaustion with H-CHO treatment during Run 2. F1, fatigue in Run 1; F2, fatigue in L-CHO treatment; F3, fatigue in H-CHO treatment.
exhaustion can therefore be confidently expected between the more markedly different very-low-carbohydrate and H-CHO doses in this study. Accordingly, establishing the absolute time point of fatigue in the L-CHO trial before the H-CHO trial enables comparisons in the metabolic environment at the point of volitional fatigue during both treatments and at the time point of H-CHO treatment corresponding to fatigue during L-CHO treatment.

Consistent with the abovementioned rationale, muscle biopsy samples were obtained in the L-CHO trial: upon cessation of Run 1, after recovery, and at volitional exhaustion during Run 2 (F2). In the H-CHO trial, three muscle biopsy samples were obtained: after recovery, at the time point coincident with fatigue in L-CHO (F2), and at the point of volitional exhaustion during the subsequent exercise bout (F3). As a result of dietary and activity standardization and because the participants ran to the point of volitional exhaustion, negligible intraindividual variability in muscle glycogen levels following Run 1 was expected between trials, as previously reported in a similar protocol (36); this was further verified by well-matched times to exhaustion during Run 1 in both trials (Results section). Thus, the sample obtained following Run 1 in the L-CHO trial merely serves to verify the expected substantial glycogen depletion from the exercise protocol, whereas the remaining samples across both trials inform the primary research questions pertaining to differences in muscle glycogen availability immediately before and during the second exercise bout.

Experimental protocol. The experimental protocol pertaining to the current study is described in further detail elsewhere (1). Each participant arrived at the laboratory at 0800 ± 1 h following an overnight fast (≥10 h). Upon arrival at the laboratory, participants completed a Profile of Mood States (POMS) questionnaire, after which a baseline urine sample was obtained. Postvoid nude body mass was recorded (Weylux 424, United Kingdom) before a 5-min resting expired gas sample was collected using the Douglas bag technique. An indwelling cannula was inserted into an antecubital vein, and a 10-mL baseline venous blood sample was collected. Participants commenced the exercise protocol with a standardized 5-min warm-up at 60% of VO\textsubscript{2max}, where speed was increased to 70% of VO\textsubscript{2max} until the point of volitional exhaustion (11 ± 1 km·h\textsuperscript{-1}). During Run 1, 1-min expired gas samples, heart rate (HR; Polar FT2, Kempele, Finland), rating of perceived exertion (RPE), and 10-mL blood samples were collected (Fig. 1). Water intake was permitted ad libitum during the familiarization trial and matched for subsequent trials (0.3 ± 0.3 L during Run 2). The point of volitional exhaustion was determined identically to the initial exercise bout. Expired gases, HR, RPE, and venous blood samples were also collected during Run 2 (Fig. 1). In the L-CHO trial, fatigue was reached after 48 ± 11 min, at which point the one remaining incision site in that trial was used to obtain a final muscle biopsy sample. Therefore, after 48 ± 11 min in the subsequent H-CHO trial, the exercise protocol was briefly (624 ± 236 s) interrupted to obtain a muscle biopsy sample at the time point coincident with fatigue during the L-CHO trial (i.e., F2), thus permitting comparison of glycogen concentrations at a matched absolute time point and rate of degradation over a matched period between the two nutritional interventions, as employed previously (36). Participants mounted the treadmill and continued to run until volitional exhaustion before the final biopsy (i.e., F3) was obtained from the remaining incision site. Body mass was subsequently recorded following the attainment of the final biopsy sample from each participant. Ambient temperature and humidity were recorded at 60-min intervals throughout the trials using a portable weather station (WS 6730; Technolime, Berlin, Germany) and were not different between the trials (20.3°C ± 0.5°C and 46% ± 2% in the L-CHO trial and 20.1°C ± 0.5°C and 47% ± 2% in the H-CHO trial). Background music was standardized between trials, and participants were unaware of the time elapsed during the exercise capacity test, with all verbal encouragement standardized.
**Solution composition.** The rates of carbohydrate (sucrose) intake in the L-CHO and H-CHO trials were 0.3 and 1.2 g·kg body mass$^{-1}·h^{-1}$, equating to a total amount of carbohydrate provided during the recovery period of 87 ± 10 and 349 ± 41 g in L-CHO and H-CHO beverages, respectively. All treatment solutions were isovolumetric (10 mL·kg body mass$^{-1}·h^{-1}$) relative to each participant's body mass (727 ± 86 mL·h$^{-1}$), thus formulating a 3% and 12% solution in L-CHO and H-CHO, respectively. Both supplements were matched for electrolyte content (sodium and potassium) and flavor. Full information pertaining to nutritional treatments is provided elsewhere (1). Owing to the design of the experiment (i.e., participants were aware of the number of biopsies planned during each run), the treatments were not blinded.

**Blood analysis.** From each 10-mL venous blood sample, 5 mL was transferred into a nonanticoagulant tube, left to clot for ~45 min at room temperature, and centrifuged at 2000g for 10 min at 4°C (Heraeus Primo R; Thermo Fisher Scientific, Loughborough, United Kingdom) for analysis of serum insulin concentrations via enzyme-linked immunosorbent assay (Merodia, Uppsala, Sweden), using a spectrophotometric plate reader (Spectrostar Nano; BMG Labtech, Ortenberg, Germany). The remaining 5 mL of each blood sample was dispensed into an ethylenediaminetetraacetic acid–treated tube and immediately analyzed for hemoglobin (Sysmex SF-3000; Sysmex Ltd., Wymbush, United Kingdom) and hematocrit (Hawksley, Lancing, United Kingdom) concentrations to determine plasma volume changes throughout the trials (14). The remaining blood was centrifuged at 2000g for 10 min at 4°C for analysis of plasma glucose, nonesterified fatty acids (NEFA), lactate, and urea using a spectrophotometric analyzer (RX Daytona; Randox Laboratories Ltd., Crumlin, United Kingdom).

**Muscle analysis.** Each muscle sample was immediately extracted from the needle biopsy and snap-frozen into liquid nitrogen, where it was subsequently dissected to remove 15–30 mg of muscle fragment before being placed in a freezer dryer (Modulyo, Edwards, United Kingdom) for ~18 h at −50°C. After removal of visible blood and connective tissue, the freeze-dried muscle samples were reduced to fine powder with agate pestle and mortar and used for the extraction and determination of phosphocreatine, creatine (Cr), and muscle glycogen concentrations. The relative concentrations of these metabolites were determined in duplicate according to previously described enzymatic methods (18,26,36), using a spectrophotometric plate reader (SpectraMax 190; Molecular Devices, USA). Glycogen was assayed by hydrolysis in 1 mol·L$^{-1}$ hydrochloric acid and determined both as acid-soluble and acid-insoluble glycogen (22). The total mixed-muscle glycogen concentration was calculated by adding the acid-soluble and acid-insoluble glycogen concentrations. All muscle metabolites were adjusted to peak total Cr (phosphocreatine + Cr) for each subject to correct for variability in blood, connective tissue, and other nonmuscle constituents between biopsies. Total glycogen concentrations are reported as millimoles of glucosyl units per kilogram of dry mass (mmol·kg dry mass$^{-1}$) to account for any measurement error associated with fluid shift during exercise and rehydration. The contribution of muscle glycogen to whole-body carbohydrate oxidation during Run 2 was estimated from the lean tissue mass of all leg muscles (6% of body mass) from a typical 72.1-kg trained individual using dual-energy x-ray absorptiometry analysis (9).

**Expired gas analysis.** Expired gas samples were collected using the Douglas bag method (Hans Rudolph, Shawnee, KS, USA), and the relative oxygen and carbon dioxide fractions were quantified by paramagnetic and infrared analyzers, respectively (Servomex, Crawborough, United Kingdom). Calculations of V$\text{O}_2$ and V$\text{CO}_2$ were used to determine carbohydrate and lipid oxidation rates (g·min$^{-1}$) using stoichiometric formulas, assuming that the contribution of protein oxidation was negligible under those conditions (24):

\[
\text{carbohydrate oxidation} = (4.210 \text{ VCO}_2) - (2.962 \text{ VO}_2)
\]

\[
\text{fatty acid oxidation} = (1.695 \text{ VO}_2) - (1.701 \text{ VCO}_2)
\]

Extramuscular carbohydrate oxidation was derived from the difference between whole-body carbohydrate oxidation (as determined from indirect calorimetry) and intramuscular carbohydrate oxidation (overall muscle glycogen degrada-

tion rate).

**Urine analysis.** Baseline urine collection for determination of hydration was assessed via freezing point depression method, using a cryoscopic osmometer (Advanced Instruments Inc., Norwood, MA, USA), and adequate hydration was assumed for osmolality values ≤900 mOsm·kg$^{-1}$ (30). During the 4-h recovery period, voided urine was collected in a vessel to determine total urine output during recovery.

**POMS questionnaire.** On the day of each trial, before exercise, participants completed a 37-item short-form POMS (POMS-SF) questionnaire (28). POMS-SF items are divided into six categories: tension, depression, anger, fatigue, confusion, and vigor. Total mood disturbance was calculated as the sum of the first five categories minus vigor.

**Statistical analysis.** *A priori* sample size estimation was conducted based on the exercise capacity data of a similar previous study (7), which showed that a sample size of N = 6 would provide 90% power to detect a 16.2-min difference in exercise capacity (using a two-tailed t-test) between two carbohydrate supplements with differing amounts. Paired t-tests were used to analyze data involving a single comparison of two level means. Where paired-difference data are deemed to be non–normally distributed on Shapiro–Wilks test, values are reported as median (range), with Wilcoxon signed rank test used to compare medians. A two-way linear mixed model with repeated measures (time × trial) was employed to identify overall differences between experimental conditions. Wherever a significant interaction effect was found, Bonferroni stepwise correction was employed to determine the location of the variance (3). Pearson product moment correlation coefficient (r) was used to explore the relationship between muscle glycogen availability at the end of recovery and...
time to exhaustion during Run 2. The incremental area under the concentration curve (iAUC) for plasma glucose and serum insulin concentrations during recovery was calculated using the method recommended by Wolever (40). Statistical procedures were performed using commercially available software (IBM SPSS version 21.0; SPSS Inc., Chicago, IL), and significance was set at an alpha level of 0.05. Unless otherwise stated, all results in the text are reported as mean ± SD, and error bars in figures are reported as confidence intervals (CI) that have been corrected to remove between-subject variance (25).

RESULTS

Exercise capacity. Run times to exhaustion in Run 1 (i.e., before intervention) were very well-matched between treatments, with a median time to exhaustion of 105 min (72–133 min) in the L-CHO trial and 105 min (75–161 min) in the H-CHO trial (P = 0.12). All participants were able to run longer during the subsequent run when more carbohydrates had been ingested during recovery, with mean run times of 48 ± 11 min in L-CHO and 80 ± 16 min in H-CHO (P < 0.001). Moreover, the magnitude of this pattern between treatments was consistent for every participant in the study (i.e., improvement of 31 ± 9 min), as represented in Figure 2.

Relative exercise intensities were also successfully standardized between the experimental treatments, with mean intensities of 69% ± 1% of VO2max in Run 1 and 69% ± 3% of VO2max in Run 2 across both treatments. These were reflected by the overall HR of 169 ± 9 and 167 ± 9 bpm recorded during L-CHO and H-CHO, respectively.

Muscle glycogen. Figure 2 illustrates muscle glycogen concentrations across both treatments. A time–trial interaction was established for total muscle glycogen concentrations (F = 9.8, P = 0.003); accordingly, there was greater muscle glycogen content at the end of recovery in the H-CHO trial than in the L-CHO trial. Despite a higher rate of glycogen degradation during Run 2 of H-CHO treatment (3.1 ± 1.5 mmol·kg dry mass⁻¹·min⁻¹) compared with the absolute fatigue time point in the L-CHO trial (1.6 ± 1.3 mmol·kg dry mass⁻¹·min⁻¹; P = 0.05), the muscle glycogen concentration at F2 was still higher in the former trial (123 ± 28 vs 72 ± 21 mmol·kg dry mass⁻¹; P < 0.01). Muscle glycogen concentrations were reduced to similar levels at the point of volitional exhaustion in both trials (Fig. 2). A significant correlation was established (r = 0.45, P = 0.045) between muscle glycogen content at the end of recovery and time to exhaustion during Run 2.

Plasma glucose and NEFA. A time–trial interaction was observed in plasma glucose during recovery (F = 8.65, P = 0.004; Fig. 3), which was associated with a higher glycemic iAUC during recovery in the H-CHO trial (299 ± 125 mmol·240 min·L⁻¹) compared with the L-CHO trial (180 ± 138 mmol·240 min·L⁻¹; P = 0.04). There were also notable differences during the subsequent run (F = 5.63, P = 0.02), with slightly lower plasma glucose concentrations in the H-CHO trial than in the L-CHO trial in the initial 30 min of exercise. No frank hypoglycemia was observed at the point of fatigue in the L-CHO trial (4.9 ± 1.1 mmol·L⁻¹) or the H-CHO trial (5.0 ± 0.9 mmol·L⁻¹).

FIGURE 2 — A. Muscle glycogen concentrations at the end of Run 1 (Fatigue 1), the end of 4-h recovery, time to exhaustion with L-CHO treatment (Fatigue 2), and time to exhaustion with H-CHO treatment (Fatigue 3). B. Mean and individual run times to exhaustion following the ingestion of L-CHO or H-CHO during 4-h recovery. Values are means ± CI. *Values different between L-CHO and H-CHO (P < 0.01).

FIGURE 3 — Plasma glucose and serum insulin concentrations during Run 1, recovery, and Run 2 with L-CHO or H-CHO treatment. Values are presented as mean ± CI. *Values different between L-CHO and H-CHO treatments (P < 0.05). F1, time to exhaustion during Run 1; F2, time to exhaustion with L-CHO treatment; F3, time to exhaustion with H-CHO treatment.
Plasma NEFA concentrations were rapidly suppressed to basal levels during recovery in the H-CHO trial but were maintained at a relatively higher level in the L-CHO trial (treatment: \( P = 0.04 \)). Upon commencement of the subsequent run, plasma NEFA concentrations were consistently elevated in the L-CHO trial compared with the H-CHO trial (treatment: \( P < 0.001 \)). An increase in NEFA concentrations from F2 to F3 was observed in the H-CHO trial (\( P = 0.008 \); Fig. 4).

**Serum insulin.** Serum insulin concentrations were significantly higher during recovery when H-CHO—as opposed to L-CHO—was ingested (\( F = 9.0, P = 0.004 \); Fig. 2). Accordingly, the insulinenic iAUC for the entire 4-h recovery period was elevated threefold when H-CHO—instead of L-CHO—was ingested (28 ± 12 vs 7 ± 3 mmol·240 min\(^{-1} \); \( P = 0.02 \)).

**Plasma lactate and urea.** Plasma lactate concentrations declined during recovery in the L-CHO trial but remained relatively elevated in the H-CHO trial (time: \( P = 0.005 \)). However, plasma lactate levels during the subsequent run (Fig. 4) were not dissimilar between the L-CHO trial and the H-CHO trial (2.5 ± 0.3 and 2.6 ± 0.2 mmol·L\(^{-1} \), respectively; \( P = 0.6 \)). Plasma concentration of urea was not different between treatments and remained at basal levels throughout the trials (5.6 ± 0.4 mmol·L\(^{-1} \) in both treatments).

**Substrate metabolism.** Whole-body carbohydrate and lipid oxidation rates were substantially different between treatments during Run 2 (\( F = 7.96, P = 0.006 \); Table 1). Although overall rates of metabolism during the repeated exercise bout were similar between treatments (L-CHO: 64.9 kJ·min\(^{-1} \); H-CHO: 66.7 kJ·min\(^{-1} \)), H-CHO ingestion resulted in lower lipid oxidation rates, compared with L-CHO ingestion (4.3 ± 2.2 vs 11.2 ± 3.5 mg·kg\(^{-1} \)·min\(^{-1} \); \( P < 0.001 \)), but higher rates of carbohydrate oxidation (44.5 ± 6.5 vs 25.2 ± 9.3 mg·kg\(^{-1} \)·min\(^{-1} \), respectively; \( P < 0.001 \)). Figure 5 illustrates that the higher rates of whole-body carbohydrate oxidation in the H-CHO trial were likely attributable to variations in glycogen rather than variations in extramuscular carbohydrate metabolism (e.g., glucose and lactate), both at the point corresponding to fatigue during L-CHO treatment (F2) and the point of absolute fatigue (F3).

**Hydration and subjective data.** Pre-exercise hydration status verified adequate fluid balance and was not different between treatments, as indicated by a urine osmolality of 496 ± 316 and 540 ± 266 mOsm·kg\(^{-1} \) with L-CHO and

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**TABLE 1.** Substrate metabolism and RER during Runs 1 and 2 with L-CHO or H-CHO treatment.

<table>
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<tr>
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<th>Run 1</th>
<th>Run 2</th>
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<tr>
<td></td>
<td>Pre 30 min 60 min 90 min</td>
<td>15 min 30 min 45 min</td>
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<tr>
<td>Carbohydrate oxidation (g·min(^{-1} ))</td>
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<tr>
<td>L-CHO 0.26 ± 0.15</td>
<td>2.20 ± 0.36 2.18 ± 0.37 1.90 ± 0.40 1.87 ± 0.72</td>
<td>1.92 ± 0.74* 1.99 ± 0.89* 1.74 ± 1.04 1.60 ± 0.79*</td>
</tr>
<tr>
<td>H-CHO 0.33 ± 0.19</td>
<td>2.59 ± 0.70 2.30 ± 0.59 2.02 ± 0.80 1.98 ± 0.73</td>
<td>2.68 ± 0.68 3.18 ± 1.06 2.81 ± 0.95 2.41 ± 0.46</td>
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<tr>
<td>Lipid oxidation (g·min(^{-1} ))</td>
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<tr>
<td>L-CHO 0.06 ± 0.06</td>
<td>0.57 ± 0.20 0.61 ± 0.21 0.76 ± 0.22 0.77 ± 0.36</td>
<td>0.77 ± 0.22* 0.71 ± 0.31* 0.56 ± 0.31 0.94 ± 0.24*</td>
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<tr>
<td>H-CHO 0.06 ± 0.07</td>
<td>0.44 ± 0.20 0.57 ± 0.23 0.66 ± 0.44 0.70 ± 0.32</td>
<td>0.24 ± 0.17 0.26 ± 0.18 0.40 ± 0.26 0.45 ± 0.16</td>
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<tr>
<td>RER</td>
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<tr>
<td>L-CHO 0.87 ± 0.12</td>
<td>0.89 ± 0.04 0.88 ± 0.04 0.85 ± 0.04 0.85 ± 0.06</td>
<td>0.85 ± 0.05 0.86 ± 0.07* 0.90 ± 0.07 0.82 ± 0.05*</td>
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<tr>
<td>H-CHO 0.90 ± 0.11</td>
<td>0.91 ± 0.04 0.89 ± 0.04 0.88 ± 0.08 0.86 ± 0.06</td>
<td>0.96 ± 0.05 0.95 ± 0.04 0.92 ± 0.05 0.91 ± 0.04</td>
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Values are presented as mean ± SD.

*Values different between L-CHO and H-CHO treatments (\( P < 0.05 \)).

†Values different at absolute fatigue (F2 vs F3) between L-CHO and H-CHO treatments (\( P < 0.05 \)).
H-CHO, respectively \((P = 0.5)\). Changes in body mass were similar \((P = 0.6)\) across both trials \((-1.2 \pm 0.6 \text{ kg to } -1.3 \pm 0.6 \text{ kg in L-CHO and H-CHO, respectively})\). Change in plasma volume was also similar \((P = 0.9)\) between the respective treatments \((2\% \pm 3\% \text{ vs } 1.8\% \pm 3\%, \text{ respectively})\). The total urine produced during recovery was 1749 \(\pm 840 \text{ mL in the L-CHO trial and 1247} \pm 613 \text{ mL in the H-CHO trial (}\(P = 0.09)\). There were no differences in any of the mood state categories in the POMS-SF \((P > 0.05)\). A significant time–trial interaction was observed for RPE \((F = 6.38, P = 0.01)\); participants’ perceived effort was significantly higher with H-CHO than with L-CHO from 15 min to F2 during Run 2 \((P < 0.05)\). Subjective ratings of gut fullness, thirst, and stomach discomfort were similar between the experimental conditions (data not shown).

**DISCUSSION**

The experimental design presented here provides novel insights into the role of muscle glycogen in fatigue by enabling both time-matched and fatigue-matched comparisons of substrate availability and utilization during the late stages of repeated exercise. Effective standardization of other relevant variables lends direct support to the hypothesis that muscle glycogen availability after recovery from prior exercise is a primary determinant of subsequent exercise capacity. From a practical perspective, after having utilized nutritional manipulation of carbohydrate availability to understand the role of glycogen, we can conclude that carbohydrate ingestion can be employed to impact repeated exercise capacity via this mechanism.

Improvement in subsequent endurance capacity with H-CHO treatment was clearly demonstrated by an increase of 31 \(\pm 9 \text{ min relative to L-CHO treatment, in agreement with one previous experiment (7) but in contrast with two others (15,41). These discrepancies may be a consequence of a number of factors. The current study, in addition to that of Betts et al. (7), included younger participants with higher \(\dot{V}O_2\text{max}\) compared with participants in previous investigations (15,41). Furthermore, we employed a familiarization trial that was identical to the main experimental procedures. These measures may be an important distinction when considering that aerobically trained individuals familiarized with exercise capacity testing may be necessary to detect small, worthwhile intervention effects (19). Moreover, subtle differences in the current experimental procedures may have contributed to accurately reaching true volitional exhaustion. Specifically, participants in the current experiment, as well as participants in the only other study reporting a dose-dependent improvement in exercise capacity with carbohydrate ingestion (7), reduced the intensity on two occasions before fatigue was accepted. Indeed, participants were able to run for 10 \(\pm 4 \text{ min from the first walk until the point of exhaustion in this study, enforcing the notion that volitional exhaustion may not have been achieved in previous investigations that did not allow these walks. Of course, other differences between protocols, such as the precise type, amount, and/or feeding frequency of the ingested carbohydrate, offer possible alternative explanations (15,41). Ingestion of 1.2 g sucrose kg\(^{-1}\) h\(^{-1}\) markedly increased muscle glycogen availability compared with the relatively low quantity of sucrose (0.3 g kg\(^{-1}\) h\(^{-1}\)). This finding is consistent with most previous studies that investigated muscle glycogen restoration with differing amounts of carbohydrate (8). In the current experiment, muscle glycogen utilization was accelerated with higher carbohydrate intake; thus, glycogenolysis was shown to be proportional to muscle glycogen concentration, as has been previously determined (2,29). Nevertheless, similar rates of muscle glycogen utilization were reported during a repeated exercise bout when differing amounts of carbohydrate were ingested during recovery (4,34). The precise reasons for these apparently discrepant findings in relation to muscle glycogen utilization may be ascribed to the use of \([13C]\) magnetic resonance spectroscopy by Berardi et al. (4) to quantify muscle glycogen degradation (i.e., wider muscle mass vs biochemical analysis of <100 mg from the *vastus lateralis*; these techniques correlated well (32)) and the type of exercise performed (i.e., cycling), which were dissimilar from the present study. Equally, the study by Tsintzas et al. (34) employed treadmill running during a nonexhaustive exercise bout (15 min) and provided lower amounts of carbohydrate than the current experiment \((0.15 \text{ vs } 0.53 \text{ g kg}^{-1} \text{ h}^{-1})\). Concurrent with our finding that muscle glycogen concentrations were reduced to similar levels at the point of volitional exhaustion across both treatments, current data suggest that muscle glycogen availability *per se* was associated with the improved restoration of endurance capacity with increased carbohydrate intake.

When interpreting the ergogenic effect of H-CHO ingestion, we must consider the brief period in this trial when exercise was interrupted to obtain a muscle biopsy sample to compare glycogen utilization at this fatigue-matching point relative to L-CHO ingestion. Muscle glycogen restoration would occur at very low rates in the absence of carbohydrate feeding \((<0.5 \text{ mmol kg}^{-1} \text{ dry mass}^{-1} \text{ min}^{-1})\). During a subsequent exercise bout at similar intensities, muscle glycogen utilization was estimated to be \(\approx 2.5 \text{ mmol kg}^{-1} \text{ dry mass}^{-1} \text{ min}^{-1}\) during treadmill running (34). Thus, any resynthesis that may have occurred during the brief interruption period \((624 \pm 236 \text{ s})\) would theoretically account for only 2 min of extended exercise. Other possibilities that may have influenced subsequent exercise capacity in H-CHO treatment include knowledge of the treatment order and the psychological impact of the resting period to obtain the muscle biopsy sample. Nevertheless, regarding the former, it was previously demonstrated that there was no placebo effect when carbohydrate was ingested during prolonged cycling and that there was a clear ergogenic effect of carbohydrate intake relative to both placebo and water ingestion (20). In relation to the psychological effect of the brief period for obtaining the muscle sample, it was apparent that participants...
were able to continue exercising during H-CHO treatment (RPE; 16 ± 1) relative to the fatigue-matching point (i.e., F2) during L-CHO treatment (20 ± 0), thus indicating that participants’ perceived effort was lower with H-CHO treatment than with L-CHO treatment before exercise was interrupted in the H-CHO trial to obtain the final biopsy sample. When considered collectively, it is reasonable to affirm that the short period for obtaining a muscle sample is unlikely to explain the 65% improvement in the capacity for subsequent exercise and that the imposed nutritional intervention may be ascribed for the ergogenic effect of H-CHO intake.

The lowering of blood glucose was more prominent in the H-CHO trial during the initial 30 min of the subsequent run, likely reflecting a transient increase in leg glucose uptake and reduced liver glucose output secondary to the increase in insulin concentrations (27). Conversely, the relatively elevated plasma glucose concentrations early during exercise in the L-CHO trial likely reflect an increased hepatic glucose output, which is predominantly supported by an increased rate of hepatic glycogenolysis (38). Thus, the increased insulminemic response during recovery in the H-CHO trial may have initially spared liver glycogenolysis such that glucose production in active muscles was possible late in exercise. These physiological responses, coupled with our finding of limited muscle glycogen restoration in the L-CHO trial, support our prior assumption that modest amounts of ingested carbohydrate will be largely sequestered by the liver due to a highly efficient first-pass hepatic extraction (10,39). It is likely that liver glycogen resynthesis was augmented in both trials owing to the presence of fructose in the sucrose solutions (13); thus, the ongoing absorption of ingested carbohydrate during H-CHO treatment is likely to contribute to the observed higher carbohydrate oxidation with this treatment. Indeed, both liver and muscle glycogen play important roles in the restoration of subsequent endurance capacity (10). Therefore, it is not unreasonable to suggest that liver glycogen availability and increased exogenous carbohydrate oxidation may have contributed to the overall effect of H-CHO treatment. Nonetheless, estimations of extramuscular carbohydrate oxidation were not different between F2 and F3 with H-CHO treatment (Fig. 5). In conjunction with the observation of an increased glycogen utilization rate with H-CHO treatment and the fact that fatigue in both treatments coincided with depletion of muscle glycogen to critically low muscle glycogen concentrations, the current findings demonstrate that the availability of muscle glycogen is a primary determinant of fatigue during a repeated exercise bout following short-term recovery.

Hypoglycemia and subsequent reduction in carbohydrate oxidation late in exercise have been proposed as major causes of fatigue during prolonged moderate-intensity to high-intensity cycling exercise (12). However, it has been consistently demonstrated that fatigue during prolonged moderate-intensity to high-intensity running is not associated with hypoglycemia (33,35,36). The latter notion was further supported by the current investigation, whereby fatigue was not associated with hypoglycemia in either treatment. Additionally, although carbohydrate oxidation during a repeated exercise bout was greater when higher amounts of carbohydrate (≥0.75 g·kg⁻¹·h⁻¹)—relative to a lower dose (≥0.25 g·kg⁻¹·h⁻¹)—were provided during recovery, no discernable differences in plasma glucose concentrations or time to exhaustion were shown (15). Indeed, fatigue during prolonged exercise was shown to be independent of carbohydrate oxidation or avoidance of hypoglycemia (11). Further support for the latter study comes from the H-CHO trial in the present investigation, where neither hypoglycemia nor a decline in carbohydrate oxidation was apparent at the cessation of exercise to explain fatigue. Thus, it can be suggested that factors other than hypoglycemia or a decline in carbohydrate oxidation rate limited the capacity for subsequent exercise.

In conclusion, the ingestion of 1.2 g carbohydrate·kg⁻¹·h⁻¹ (compared with the ingestion of 0.3 g·kg⁻¹·h⁻¹) during a 4-h recovery from an initial exhaustive exercise bout increased muscle glycogen availability before a repeated exercise bout. In concordance, the capacity for repeated exercise was improved in a dose-dependent manner. The rate of glycogen utilization was accelerated in the H-CHO trial during the repeated exercise bout, and fatigue was associated with glycogen depletion to critically low levels in both treatments. The extended run time to fatigue expected with increasing carbohydrate intake is attributable to increased muscle glycogen repletion during recovery and, therefore, the availability of this substrate during Run 2.

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REFERENCES


