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Citation for published version:
Alghannam, A, Jedrzejewski, D, Tweddle, MG, Gribble, H, Bilzon, J, Thompson, D, Tsintzas, K & Betts, JA 2016, 'Impact of muscle glycogen availability on the capacity for repeated exercise in man', Medicine & Science in Sports & Exercise, vol. 48, no. 1, pp. 123-131. https://doi.org/10.1249/MSS.0000000000000737

DOI:
10.1249/MSS.0000000000000737

Publication date:
2016

Document Version
Peer reviewed version

Link to publication

University of Bath

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Download date: 13. May. 2019

Impact of muscle glycogen availability on the capacity for repeated exercise in man

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Running head: Carbohydrate feeding, muscle glycogen and repeated exercise

30 ## Abstract

31

32 **Purpose**: To examine whether muscle glycogen availability is associated with fatigue

33 in a repeated exercise bout following short-term recovery.

34

35 **Methods:** Ten endurance-trained individuals underwent two trials in a repeated

36 measures design, each involving an initial run to exhaustion at 70% $\dot{V}O_{2max}$ (Run-1)

37 followed by a 4-h recovery and a subsequent run to exhaustion at 70% $\dot{V}O_{2max}$ (Run-

38 2). A low-carbohydrate (L-CHO; 0.3 g·kg BM^{-1}·h^{-1}) or high-carbohydrate (H-CHO;

39 1.2 g·kg BM^{-1}·h^{-1}) beverage was ingested at 30-min intervals during recovery. Muscle

40 biopsies were taken upon cessation of Run-1, post-recovery and fatigue during Run-2

41 in L-CHO (F2). In H-CHO, the muscle biopsies were obtained post-recovery, the time

42 point coincident with fatigue in L-CHO (F2) and the point of fatigue during the

43 subsequent exercise bout (F3).

44

45 **Results:** Run-2 was more prolonged for every participant in H-CHO (80±16 min)

46 than L-CHO (48±11 min; $p< 0.001$). Muscle glycogen concentrations were higher at

47 the end of recovery in H-CHO (269±84 mmol·kg dm^{-1}) *versus* L-CHO (157±37

48 mmol·kg dm^{-1}; $p= 0.001$). The rate of muscle glycogen degradation during Run-2 was

49 higher in H-CHO (3.1±1.5 mmol·kg dm^{-1}·min^{-1}) than L-CHO (1.6±1.3 mmol·kg dm^{-}

50 ^{1}·min^{-1}; $p= 0.05$). The concentration of muscle glycogen was higher in H-CHO than

51 L-CHO at F2 (123±28 mmol·kg dm^{-1}; $p< 0.01$) but no differences were observed

52 between treatments at the respective points of exhaustion (78±22 *versus* 72±21

53 mmol·kg dm^{-1}·min^{-1}; H-CHO and L-CHO, respectively).

54

Conclusion: Increasing carbohydrate intake during short-term recovery accelerates glycogen repletion in previously exercised muscle 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.

Keywords: Nutrition, metabolism, performance, sucrose

64 ## Introduction

65 Endurance capacity during an initial prolonged exercise bout is primarily reliant upon
66 pre-exercise glycogen availability, such that muscle glycogen content exhibits a direct
67 positive correlation with exercise time to exhaustion (6, 34, 36). Similarly, muscle
68 glycogen repletion can impact the time required to recover functional capacity, with
69 carbohydrate intake accelerating both glycogen resynthesis and restoration of exercise
70 capacity relative to when no carbohydrate is ingested (16, 17, 23). Furthermore,
71 carbohydrate ingestion rate exhibits a dose-dependent relationship with the rate of
72 muscle glycogen resynthesis until a threshold of ingesting ≈1.2 $g \cdot kg\ BM^{-1} \cdot h^{-1}$ (8). It is
73 therefore logical to postulate that increasing carbohydrate intakes might also exhibit a
74 dose-dependent relationship with the restoration of exercise capacity following short-
75 term recovery. However, while some information is available pertaining to muscle
76 glycogen metabolism during a subsequent exercise bout (4, 9, 35), it remains merely
77 an assumption that muscle glycogen availability is an important determinant of
78 fatigue during a second bout of exercise following short-term recovery.

79

80 Based on the few studies to have examined the relationship between carbohydrate
81 ingestion rate in recovery and restoration of exercise capacity, most report no
82 consistent pattern (7, 15, 41), with only one reporting a dose-dependent relationship
83 (7). Notwithstanding that the aforementioned studies did not provide any glycogen
84 data, there is some evidence that glycogen resynthesis (in particular liver glycogen) is
85 an important determinant of endurance capacity following short-term recovery (10).
86 This is understandable given that liver glycogen content is preferentially
87 resynthesized over muscle glycogen when modest amounts of carbohydrate (≈0.3

88 g·kg $BM^{-1}·h^{-1}$) are ingested following an initial exhaustive exercise bout (10).
89 Conversely, the capacity for repeated exercise has also been dissociated from skeletal
90 muscle glycogen availability in other studies (4, 7, 9). It is therefore possible that
91 fatigue during repeated exercise may manifest differently from an initial prolonged
92 exercise bout, and the availability of muscle glycogen may not be the primary cause
93 of fatigue during subsequent exercise under all conditions. Accordingly, there is an
94 outstanding need for improved understanding about the relative importance of muscle
95 glycogen availability in offsetting fatigue during a repeated exercise bout as opposed
96 to an initial bout, with implications for the optimal carbohydrate feeding strategy in
97 recovery to maximize not only glycogen resynthesis but also restoration of exercise
98 capacity.
99
100 To this end, the current study nutritionally manipulated carbohydrate availability
101 during short-term recovery to examine metabolic and ergogenic outcomes during
102 subsequent exercise. Specifically, we sought to examine whether muscle glycogen
103 availability is associated with fatigue in a repeated exercise bout following short-term
104 recovery. Comparisons were therefore made between a low-carbohydrate (L-CHO)
105 supplement sufficient only to restore hepatic glycogen with minimal rates of muscle
106 glycogen resynthesis (10), and a high-carbohydrate (H-CHO) supplement designed to
107 elicit high rates of muscle glycogen resynthesis (37). It was hypothesized that the
108 extended run time to fatigue expected with increasing carbohydrate intake would be
109 explained by a proportional acceleration of muscle glycogen resysnthesis during
110 recovery and thus greater glycogen availability during repeated exercise.
111

112 # Materials and Methods

113 ## Participants

114 Nine healthy recreationally active men and one woman participated in the study. The
115 characteristics of this sample were: age 21 ± 1 years; body mass (BM) 72.5 ± 8.2 kg;
116 height 180 ± 9 cm; $\dot{V}O_{2max}$ 61 ± 6 ml·kg^{-1}·min^{-1}; weekly exercise duration 5 ± 3 h.
117 The participants were informed about the possible risks and discomforts involved
118 before giving their voluntary consent to take part. The current study has been
119 approved by the local National Health Services Research Ethics Committee (Ref:
120 09/H0101/82) with a controlled clinical trial number: ISRCTN87937960.

121

122 ## Preliminary measurements

123 Participants undertook preliminary testing on two separate occasions. The first
124 preliminary visit included the determination of each participant's submaximal ($\dot{V}O_2$)
125 and maximal ($\dot{V}O_{2max}$) oxygen uptakes (31) on a motorized treadmill (Ergo ELG70,
126 Woodway, Weil am Rhein, Germany). The data acquired from these tests were then
127 employed to calculate the treadmill speeds that elicit 60 % and 70 % of $\dot{V}O_{2max}$. The
128 second visit (familiarization trial) was completed at least two weeks prior to the main
129 trials and required each participant to undergo the exercise protocol used in the main
130 trials (described below) without any tissue or venous blood collection, and
131 participants only ingested water at similar intervals to nutrient provision during the
132 main trials (Figure 1)._This trial was aimed to accustom the participants to the
133 experimental procedures and apparatus in addition to fully familiarize with running to
134 the point of volitional exhaustion and thereby diminish any learning/trial-order effects

135 (Run-1 and Run-2 times to exhaustion were 103 ± 17 min and 36 ± 9 min,

136 respectively). Expired gas samples were collected during this visit to confirm the

137 estimated relative speeds that corresponded to the required intensity during the main

138 trials, with any adjustments applied accordingly.

139

140 Experimental design

141 Each participant performed two main trials in a repeated measures experimental

142 design interspersed by an interval of ≥ 2 weeks. A weighed dietary record was

143 completed 48 h preceding the familiarization trial, and was subsequently repeated

144 prior to the commencement of the main trials (2638 ± 708 $kcal \cdot d^{-1}$; 55 ± 5 %

145 carbohydrate; 17 ± 3 % fat; 28 ± 4 % protein). Participants were provided with a

146 standardized meal (760 kcal; 57 % carbohydrate; 24 % Protein; 19 % fat) in the

147 evening (12 ± 1 h) before the familiarization trial and replicated this prior to each

148 main trial. Participants were also requested to abstain from alcohol consumption and

149 refrain from strenuous physical activity for 48 h pre-trial, with any light exercise

150 recorded and matched during the period of standardization of lifestyle for ensuing

151 trials.

152

153 The main trials required participants to run to the point of volitional exhaustion (Run-

154 1) at an intensity of 70 % $\dot{V}O_{2max}$ followed by a 4 h recovery period, where

155 participants ingested a low carbohydrate (L-CHO) or a high carbohydrate (H-CHO)

156 supplement. Following recovery, a second run to exhaustion (Run-2) at the same

157 exercise intensity (i.e. 70 % $\dot{V}O_{2max}$) was undertaken by each participant to assess

158 restoration of exercise capacity. As has been successfully applied in previous studies

159 that have contrasted relative and absolute fatigue points to understand fatigue
160 mechanisms in relation to running (34) and cycling (12), trial order required L-CHO
161 to be completed first. Previous data (7) has reported that the restoration of exercise
162 capacity can be dose-dependent with ingestion of moderate-high *versus* high
163 carbohydrate during short-term recovery. Differences in exercise time to exhaustion
164 can therefore be confidently expected between the more markedly different very low
165 *versus* high carbohydrate doses in this study. Accordingly, establishing the absolute
166 time-point of fatigue in L-CHO trials prior to H-CHO trials enables comparisons in
167 the metabolic environment both at the point of volitional fatigue in both treatments
168 and at the time point in the H-CHO treatment that corresponds to fatigue during L-
169 CHO treatment.

170

171 Consistent with the above rationale, muscle biopsy samples were obtained in L-CHO
172 trial: upon cessation of Run-1; post-recovery; and volitional exhaustion during Run-2
173 (F2). In the H-CHO trial, the three muscle biopsy samples were obtained: post-
174 recovery; the time point coincident with fatigue in L-CHO (F2); and the point of
175 volitional exhaustion during the subsequent exercise bout (F3). As a result of the
176 dietary and activity standardization, and the fact the participants ran to the point of
177 volitional exhaustion, negligible intra-individual variability in muscle glycogen levels
178 following Run-1 were expected between trials, as previously reported in a similar
179 protocol (34) and this was further verified by the well-matched times to exhaustion
180 during Run-1 in both trials (results section). Thus, the sample obtained following
181 Run-1 in L-CHO merely serves to verify the expected substantial glycogen depletion
182 from the exercise protocol, whilst the remaining samples across both trials inform the

183 primary research questions pertaining to differences in muscle glycogen availability
184 immediately prior to and during the second exercise bout.
185
186 **Experimental protocol**
187 The experimental protocol pertaining to the current study is described in further detail
188 elsewhere (1). Each participant arrived to the laboratory at 08:00 ± 1 h following an
189 overnight fast (≥ 10 h). Upon arrival at the laboratory, participants completed a profile
190 of mood state (POMS) questionnaire, before a baseline urine sample was obtained.
191 Post-void nude body mass (BM) was then recorded (Weylux 424, UK) before a 5 min
192 resting expired gas sample was collected using the Douglas bag technique. An
193 indwelling cannula was inserted into an antecubital vein and a 10 ml baseline venous
194 blood sample was collected. Participants commenced the exercise protocol with a
195 standardized 5 min warm-up at 60 % $\dot{V}O_{2max}$, where speed was then increased to 70%
196 $\dot{V}O_{2max}$ until the point of volitional exhaustion (11 ± 1 km·h^{-1}). During Run-1, one
197 minute expired gas samples, heart rate (HR; Polar FT2, Kempele, Finland), ratings of
198 perceived exertion (RPE), and 10 ml blood samples were collected (Figure 1). Water
199 intake was permitted *ad libitum* during the familiarization trial (0.5 ± 0.3 L during
200 Run-1) and then matched for subsequent trials. To accurately gauge relative levels of
201 fatigue, participants were permitted to reduce the intensity (walking at 4.4 km·h^{-1}) for
202 2 min intervals on two occasions when they indicated that they could not maintain the
203 prescribed intensity, followed by a return to the treadmill speed equivalent to 70 %
204 $\dot{V}O_{2max}$. Only on the third occasion that participants indicated that they were unable
205 to run at the prescribed speed was volitional exhaustion accepted. Immediately
206 following Run-1 in L-CHO trial, participants rested on an adjacent bed in a semi-

207 supine position while ≈80 mg of muscle was obtained from the *vastus lateralis* by
208 percutaneous needle biopsy technique (5) from a 3-5 mm incision made prior to
209 exercise at the anterior aspect of the thigh using a surgical blade under local anesthetic
210 (1 % lidocaine; Hameln Pharmaceuticals Ltd., Brockworth, UK). Thereafter, the first
211 bolus of prescribed solution was immediately provided and recovery time
212 commenced, before nude BM mass was recorded to assess hydration status through
213 percentage change in mass (with body mass adjusted for the ingested bolus). The
214 remaining seven aliquots of prescribed solution were provided at 30 min intervals
215 (Figure 1). Participants were permitted 15 min to consume each volume, and
216 subjective measures of stomach discomfort, gut fullness and thirst were recorded
217 following the ingestion of each bolus using adapted Borg scales (1). Expired gas
218 followed by venous blood samples were collected hourly prior to fluid provision.
219 Furthermore, urine output was collected throughout the 4 h recovery period.
220 Approximately 3 h 40 min into recovery, two (in L-CHO trial) or three (in H-CHO
221 trial) 3-5 mm incisions were made proximally on the same leg at least 3 cm apart
222 followed by obtaining a muscle biopsy sample at the end of recovery (with the
223 remaining incisions dressed for easy access at later sampling points), with the order of
224 dominant/non-dominant legs for muscle biopsy sampling being counterbalanced
225 between the main trials. Nude BM was recorded at the end of recovery, and
226 participants initiated a standardized warm-up before running at 70 % $\dot{V}O_{2max}$ to
227 volitional exhaustion. As for Run-1, water intake was permitted *ad libitum* during the
228 familiarization trial and matched for subsequent trials (0.3 ± 0.3 L during Run-2).
229 Reaching the point of volitional exhaustion was determined in an identical manner to
230 the initial exercise bout. Expired gases, HR, RPE and venous blood samples were also
231 collected during Run-2 (Figure 1). In the L-CHO trial fatigue was reached after 48 ±

232 11 min, at which point the one remaining incision site in that trial was used to obtain a
233 final muscle biopsy. Therefore, after 48 ± 11 min in the subsequent H-CHO trial, the
234 exercise protocol was briefly (624 ± 236 seconds) interrupted to obtain a muscle
235 biopsy at the time point coincident with fatigue during L-CHO trial (i.e. F2) – thus
236 permitting comparison of glycogen concentrations at a matched absolute time-point
237 and rate of degradation over a matched period between the two nutritional
238 interventions, as employed previously (34). Participants then mounted the treadmill
239 and continued to run until volitional exhaustion before the final biopsy (i.e. F3) was
240 obtained from the remaining incision site. BM was subsequently recorded following
241 the attainment of the final biopsy from each participant. Ambient temperature and
242 humidity were recorded at 60 min intervals throughout the trials using a portable
243 weather station (WS 6730; Technoline, Berlin, Germany) and were not different
244 among the trials: 20.3 ± 0.5 and 20.1 ± 0.5°C; and 46 ± 2 and 47 ± 2 % in L-CHO and
245 H-CHO trials, respectively. Background music was standardized between trials and
246 participants were unaware of the time elapsed during the exercise capacity test, with
247 all verbal encouragement standardized.

248

249 Solution composition

250 The rates of carbohydrate (sucrose) intake in the L-CHO and H-CHO trials were 0.3
251 $g \cdot kg\ BM^{-1} \cdot h^{-1}$ and 1.2 $g \cdot kg\ BM^{-1} \cdot h^{-1}$, equating to a total amount of carbohydrate
252 provided during the recovery period of 87 ± 10 g and 349 ± 41 g in L-CHO and H-
253 CHO beverages, respectively. All treatment solutions were isovolumetric (10 $ml \cdot kg$
254 $BM^{-1} \cdot h^{-1}$) relative to each participant's BM (727 ± 86 $ml \cdot h^{-1}$), thus formulating a 3 %
255 and 12 % solution in L-CHO and H-CHO respectively. Both supplements were

256 matched for their electrolyte content (sodium and potassium) and were flavor
257 matched. Full information pertaining to the nutritional treatments is provided
258 elsewhere (1). Owing to the design of the experiment (i.e. participants were aware of
259 the number of biopsies planned during each run), the treatments were not blinded.

260

261 **Blood analysis**

262 From each 10 ml venous blood sample, 5 ml was transferred into a non-anticoagulant
263 tube and left to clot for ≈45-min at room temperature before being centrifuged at 2000
264 xg for 10 min at 4ºC (Heraeus Primo R; Thermo Fisher Scientific, Loughborough,
265 UK) for the analysis of serum insulin concentrations via enzyme-linked
266 immunosorbent assay (ELISA; Mercodia, Uppsala, Sweden) using a
267 spectrophotometric plate reader (Spectrostar Nano, BMG Labtech, Ortenberg,
268 Germany). The remaining 5 ml of each blood sample was dispensed into a
269 ethylenediaminetetraacetic acid (EDTA) treated tube and was immediately analyzed
270 for hemoglobin (Sysmex SF-3000 Sysmex Ltd., Wymbush, UK) and hematocrit
271 (Hawksley, Lancing, UK) concentrations for the determination of plasma volume
272 changes throughout the trials (14). The remaining blood was then spun for
273 centrifugation under 2000 xg for 10 min at 4ºC for the analysis of plasma glucose,
274 non-esterified fatty acids, lactate and urea using a spectrophotometric analyzer (RX
275 Daytona, Randox Laboratories Ltd., Crumlin, UK).

276

277 **Muscle analysis**

278 Each muscle sample was immediately extracted from the needle biopsy and snap-
279 frozen into liquid nitrogen, where it was subsequently dissected to remove 15-30 mg

280 of muscle fragment prior to being placed in a freeze-dryer (Modulyo, Edwards, UK)
281 for ≈18 hours at -50°C. After removal of visible blood and connective tissue, the
282 freeze-dried muscle samples were then reduced to fine powder using an agate pestle
283 and mortar and used for the extraction and determination of phosphocreatine (PCr),
284 creatine (Cr) and muscle glycogen concentrations. The relative concentrations of
285 these metabolites were determined in duplicate according to enzymatic methods
286 previously described (18, 26, 34) using a spectrophotometric plate reader
287 (SpectraMax 190, Molecular Devices, USA). Glycogen was assayed by hydrolysis in
288 1 mol/l hydrochloric acid (HCl) and was determined both as acid-soluble and acid-
289 insoluble glycogen (22). The total mixed-muscle glycogen concentration was
290 calculated by adding the acid-soluble and acid-insoluble glycogen concentrations. All
291 muscle metabolites were adjusted to peak total Cr (PCr+Cr) for each subject to correct
292 for variability in blood, connective tissue, and other non-muscle constituents between
293 biopsies. Total glycogen concentrations are reported as mmol glucosyl units per
294 kilogram of dry mass (mmol·kg dm^{-1}) to account for any measurement error
295 associated with fluid shift during exercise and rehydration. The contribution of muscle
296 glycogen towards whole-body carbohydrate oxidation during Run-2 was estimated
297 from lean tissue mass of all leg muscle (6 % of body mass) from a typical 72.1 kg
298 trained individual using dual-energy X-ray absorptiometry (DXA) analysis (9).
299

300 ## Expired gas analysis

301 Expired gas samples were collected using the Douglas bag method (Hans Rudolph,
302 Shawnee, KS, USA), and the relative oxygen and carbon dioxide factions were
303 quantified by paramagnetic and infrared analyzers, respectively (Servomex,

304 Crowborough, UK). The calculations of $\dot{V}O_2$ and $\dot{V}CO_2$ were then used for the
305 determination of carbohydrate and lipid oxidation rates (g·min^{-1}) using stoichiometric
306 formulae, assuming that the contribution of protein oxidation was negligible under
307 those conditions (24):

308 $$\text{Carbohydrate Oxidation} = (4.210 \cdot \dot{V}CO_2) - (2.962 \cdot \dot{V}O_2)$$

309 $$\text{Fatty Acid Oxidation} = (1.695 \cdot \dot{V}O_2) - (1.701 \cdot \dot{V}CO_2)$$

310 Extra-muscular carbohydrate oxidation was then derived from the difference between
311 whole-body carbohydrate oxidation as determined from indirect calorimetry and
312 intramuscular carbohydrate oxidation (overall muscle glycogen degradation rates).

313

314 Urine analysis

315 Baseline urine collection to determine hydration was assessed via freezing point
316 depression method by using a cryoscopic osmometer (Advanced Instruments, Inc,
317 Norwood, MA, USA) and adequate hydration was assumed for osmolality values ≤
318 900 mOsm·kg^{-1} (30). During the 4 h recovery period, the voided urine was collected
319 in a vessel for the determination of total urine output during recovery.

320

321 POMS questionnaire

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

327

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 difference in exercise capacity of 16.2 min 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 were deemed non-normally distributed by Shaprio-Wilk test, values are reported as median (range), with Wilcoxon signed rank test being used to compare medians. A two-way linear mixed model with repeated measures (time x trial) was employed to identify overall differences between experimental conditions. Wherever a significant interaction effect was found, a Bonferroni step-wise 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. Incremental area under the concentration curve (iAUC) for plasma glucose and serum insulin concentrations during the recovery were 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 were reported in text as the mean ± standard deviation (SD) of the mean and the error bars depicted in figures are confidence intervals (CI) that have been corrected to remove between-subject variance (25).

350

351 # Results

352 **Exercise capacity**

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

361

362 Relative exercise intensities were also successfully standardized between the
363 experimental treatments and averaged 69 ± 1 % $\dot{V}O_{2max}$ in Run-1 and 69 ± 3 %
364 $\dot{V}O_{2max}$ in Run-2 across both treatments. These were reflected by the overall heart
365 rates of 169 ± 9 and 167 ± 9 beats·min^{-1} recorded during L-CHO and H-CHO,
366 respectively.

367

368 **Muscle glycogen**

369 Figure 2 illustrates muscle glycogen concentrations across both treatments. A time x
370 trial interaction was established for total muscle glycogen concentrations (F= 9.8; p=
371 0.003) and accordingly there was greater muscle glycogen content at the end of
372 recovery in H-CHO than L-CHO. Despite a higher rate of glycogen degradation
373 during Run-2 in the H-CHO treatment (3.1 ± 1.5 mmol·kg dm^{-1}·min^{-1}), when

374 compared to the absolute fatigue time point in L-CHO trial (1.6 ± 1.3 mmol·kg dm^{-1}

375 $\cdot min^{-1}$ (p= 0.05) the muscle glycogen concentration at F2 was still higher in the

376 former trial (123 ± 28 mmol·kg dm^{-1} *versus* 72 ± 21 mmol·kg dm^{-1}; p< 0.01). Muscle

377 glycogen concentrations were reduced to similar levels at the point of volitional

378 exhaustion in both trials (Figure 2). A significant correlation was established (r= 0.45;

379 p= 0.045) between muscle glycogen content at the end of recovery and time to

380 exhaustion during Run-2.

381

382 **Plasma glucose and NEFA**

383 A time x trial interaction was observed in plasma glucose during recovery (F= 8.65;

384 p= 0.004; Figure 3), which was associated with a higher glycemic iAUC in H-CHO

385 (299 ± 125 mmol·240 $min \cdot l^{-1}$) during recovery than L-CHO (180 ± 138 mmol·240

386 $min \cdot l^{-1}$; p= 0.04). There were also notable differences during the subsequent run (F=

387 5.63; p= 0.02), with slightly lower plasma glucose concentrations in H-CHO than L-

388 CHO in the initial 30 min of exercise. No frank hypoglycemia was observed at the

389 point of fatigue in L-CHO (4.9 ± 1.1 $mmol \cdot l^{-1}$) or H-CHO (5.0 ± 0.9 $mmol \cdot l^{-1}$).

390

391 Plasma NEFA concentrations were rapidly suppressed to basal levels during recovery

392 in H-CHO while maintained at a relatively higher level in the L-CHO trial (treatment:

393 p= 0.04). Upon commencement of the subsequent run, plasma NEFA were

394 consistently elevated in L-CHO when compared to H-CHO (treatment: p< 0.001). An

395 increase in NEFA concentrations from F2 to F3 was observed in H-CHO trial (p=

396 0.008, Figure 4).

397

398 **Serum insulin**

399 Serum insulin concentrations were significantly higher during recovery when H-CHO
400 was ingested as opposed to L-CHO (F= 9.0; *p*= 0.004; Figure 2). Accordingly, the
401 insulinemic iAUC for the entire 4-h recovery period was elevated threefold when H-
402 CHO was ingested when compared to L-CHO (28 ± 12 *versus* 7 ± 3 nmol·240 min·l^{-1};
403 *p*= 0.02).

404

405 **Plasma lactate and urea**

406 Plasma lactate concentrations declined during recovery in L-CHO but remained
407 relatively elevated in H-CHO (time: *p*= 0.005). However, plasma lactate levels during
408 the subsequent run (Figure 4) were not dissimilar between in L-CHO and H-CHO (2.5
409 ± 0.3 and 2.6 ± 0.2 mmol·l^{-1}, respectively; *p*= 0.6). The plasma concentration of urea
410 was not different between treatments and remained at basal levels throughout trials
411 (5.6 ± 0.4 mmol·l^{-1} in both treatments).

412

413 **Substrate metabolism**

414 Whole-body carbohydrate and lipid oxidation rates were substantially different
415 between treatments during Run-2 (F= 7.96; *p*= 0.006; Table 1). Although overall rates
416 of metabolism during the repeated exercise bout were similar between treatments (L-
417 CHO = 64.9 kj·min^{-1}; H-CHO = 66.7 kj·min^{-1}), H-CHO ingestion resulted in lower
418 lipid oxidation rates than L-CHO (4.3 ± 2.2 vs. 11.2 ± 3.5 mg·kg^{-1}·min^{-1}; *p*< 0.001)
419 but higher rates of carbohydrate oxidation (44.5 ± 6.5 vs. 25.2 ± 9.3 mg·kg^{-1}·min^{-1},
420 respectively; *p*<0.001). Figure 5 illustrates that the higher rates of whole body
421 carbohydrate oxidation in H-CHO trial were likely attributable to variations in

422 glycogen rather than extra-muscular carbohydrate metabolism (e.g. glucose and
423 lactate), both at the point corresponding to fatigue with L-CHO (F2) and the point of
424 absolute fatigue (F3).

425

426 **Hydration and subjective data**

427 Pre-exercise hydration status verified adequate fluid balance and was not different
428 between treatments, as indicated by urine osmolality of 496 ± 316 and 540 ± 266
429 mOsm.kg^{-1} in L-CHO and H-CHO, respectively (p= 0.5). Changes in BM were
430 similar (p= 0.6) across both trials (-1.2 ± 0.6 and -1.3 ± 0.6 kg in L-CHO and H-CHO,
431 respectively). The change in plasma volume was also similar (p= 0.9) between the
432 respective treatments (2 ± 3 vs. 1.8 ± 3 %, respectively). The total urine produced
433 during recovery was 1749 ± 840 ml in L-CHO and 1247 ± 613 ml in H-CHO trials
434 (p= 0.09). There were no differences in any of the mood state categories in the
435 POMS-SF (p> 0.05). A significant time x trial interaction was observed in RPE (F=
436 6.38; p= 0.01); participants' perceived effort was significantly lower in H-CHO than
437 L-CHO from 15 min until F2 during Run-2 (p< 0.05). Subjective ratings of gut
438 fullness, thirst, and stomach discomfort were similar between the experimental
439 conditions (data not shown).

440

441 # Discussion

442 The experimental design presented here provides novel insight regarding the role of
443 muscle glycogen in fatigue by enabling both time- and fatigue-matched comparisons
444 of substrate availability and utilization during the late stages of repeated exercise.
445 Effective standardization of other relevant variables lends direct support to the

446 hypothesis that muscle glycogen availability after recovery from prior exercise is
447 indeed a primary determinant of subsequent exercise capacity. From a practical
448 perspective, having utilized nutritional manipulation of carbohydrate availability to
449 understand the role of glycogen, it can also therefore be concluded that carbohydrate
450 ingestion can be employed to impact repeated exercise capacity via this mechanism.

451

452 The improvement in subsequent endurance capacity with H-CHO treatment was
453 clearly demonstrated by an increase of 31 ± 9 min relative to L-CHO, which is in
454 agreement with one previous experiment (7) but in contrast with two others (15, 41).
455 These discrepancies may be a consequence of a number of factors. The current study
456 in addition to that of Betts et al. (7) included younger participants with higher $\dot{V}O_{2max}$
457 than those used in previous investigations (15, 41). Furthermore, we employed a
458 familiarization trial that was identical to the main experimental procedures. These
459 measures may be an important distinction when considering that aerobically trained
460 individuals who are familiarized with exercise capacity testing may be necessary to
461 detect small, worthwhile intervention effects (19). Moreover, subtle differences in the
462 current experimental procedures may have contributed to accurately reaching true
463 volitional exhaustion. Specifically, participants in the current experiment, as well as
464 the only other study reporting a dose-dependent improvement in exercise capacity
465 with carbohydrate ingestion (7), reduced the intensity on two occasions before fatigue
466 was accepted. Indeed, participants were able to run for 10 ± 4 min from the first walk
467 until the point of exhaustion in this study, enforcing the notion that volitional
468 exhaustion may not have been achieved in previous investigations that did not allow
469 these walks. Of course, other differences between protocols such as the precise type,

470 amount, and/or feeding frequency of the ingested carbohydrate offer possible
471 alternative explanations (15, 41).

472

473 Ingestion of 1.2 g sucrose·kg^{-1}·h^{-1} markedly increased muscle glycogen availability
474 compared to the relatively low quantity of sucrose (0.3 g·kg^{-1}·h^{-1}). This finding is
475 consistent with most previous studies investigating muscle glycogen restoration with
476 differing amounts of carbohydrate (8). In the current experiment, muscle glycogen
477 utilization was accelerated with higher carbohydrate intake and thus glycogenolysis
478 was shown to be proportional to muscle glycogen concentration, as has previously
479 been determined (2, 29). Nevertheless, similar rates of muscle glycogen utilization
480 were reported during a repeated exercise bout when differing amounts of
481 carbohydrate were ingested during recovery (4, 35). The precise reasons for these
482 apparently discrepant findings in relation to muscle glycogen utilization may be
483 ascribed to the use of ^{13}C-magnetic resonance spectroscopy by Berardi et al. (2006) to
484 quantify muscle glycogen degradation (i.e. wider musculature *versus* biochemical
485 analysis of <100 mg from the *vastus lateralis*; although these techniques correlate
486 well (32)) and the type of exercise performed (i.e. cycling) that were dissimilar from
487 the present study. Equally, the study by Tsintzas et al. (2003) employed treadmill
488 running during a non-exhaustive exercise bout (15 min) and provided lower amounts
489 of carbohydrate to the current experiment (0.15 g·kg^{-1}·h^{-1} *versus* 0.53 g·kg^{-1}·h^{-1}).
490 Concurrent with our finding that muscle glycogen concentrations were reduced to
491 similar levels at the point of volitional exhaustion across both treatments, the current
492 data suggest that muscle glycogen availability *per se* was associated with the
493 improved restoration of endurance capacity with increased carbohydrate intake.

494

495 When interpreting the ergogenic effect with H-CHO ingestion, it is important to
496 consider the brief period where exercise was interrupted in this trial to obtain a muscle
497 biopsy sample to compare glycogen utilization at this fatigue-matching point relative
498 to L-CHO. Muscle glycogen restoration would occur at very low rates in the absence
499 of carbohydrate feeding (≈0.5 mmol·kg dry mass^{-1}·min^{-1} (21)). During a subsequent
500 exercise bout at similar intensities, muscle glycogen utilization was estimated to be
501 ≈2.5 mmol·kg dry mass^{-1}·min^{-1} during treadmill running (35). Thus, any resynthesis
502 that may have occurred during the brief interruption period (624 ± 236 seconds)
503 would theoretically account for only 2 min of extended exercise. Other possibilities
504 that may have influenced subsequent exercise capacity in H-CHO treatment include
505 knowledge of the treatment order and the psychological impact of resting period to
506 obtain the muscle biopsy. Nevertheless, regarding the former, it was previously
507 demonstrated that there was no placebo effect when carbohydrate was ingested during
508 prolonged cycling, and that there was a clear ergogenic effect with carbohydrate
509 intake relative to both a placebo and water ingestion (20). In relation to the
510 psychological effect of the brief period to obtain the muscle sample, it was apparent
511 that participants were able to continue exercising during H-CHO (RPE; 16 ± 1)
512 relative to the fatigue-matching point (i.e. F2) in L-CHO (20 ± 0) and thus indicating
513 that participants' perceived effort was lower in H-CHO than L-CHO treatment before
514 exercise was interrupted in H-CHO to obtain the final biopsy sample. When
515 considered collectively, it is reasonable to affirm that the short period to obtain a
516 muscle sample is unlikely to explain the 65 % improvement in the capacity for
517 subsequent exercise and that the imposed nutritional intervention may be ascribed for
518 the ergogenic effect with H-CHO intake.

519

520 The lowering of blood glucose was more prominent in H-CHO during the initial 30
521 min of the subsequent run, likely reflecting a transient increase in leg glucose uptake
522 and reduced liver glucose output secondary to the increase in insulin concentrations
523 (27). Conversely, the relatively elevated plasma glucose concentrations early in
524 exercise in the L-CHO trial likely reflect an increased hepatic glucose output, which is
525 predominantly supported by an increased rate of hepatic glycogenolysis (38). Thus,
526 the increased insulinemic response during recovery in H-CHO may have initially
527 spared liver glycogenolysis such that glucose production to the active muscles was
528 possible late in exercise. These physiological responses coupled with our finding of
529 limited muscle glycogen restoration in L-CHO supports our prior assumption that the
530 modest amounts of ingested carbohydrate will be largely sequestered by the liver due
531 to highly efficient first pass hepatic extraction (10, 39). It is likely that liver glycogen
532 resynthesis was augmented in both trials owing to the presence of fructose in the
533 sucrose solutions (13) and thus the ongoing absorption of the ingested carbohydrate in
534 H-CHO treatment is likely to contribute to the observed higher carbohydrate
535 oxidation with this treatment. Indeed, both liver and muscle glycogen have an
536 important role in restoration of subsequent endurance capacity (10). Therefore, it is
537 not unreasonable to suggest that liver glycogen availability and increased exogenous
538 carbohydrate oxidation may have contributed to the overall effect in the H-CHO
539 treatment. Nonetheless, estimations of extra-muscular carbohydrate oxidation were
540 not different between F2 and F3 in the H-CHO treatment (Figure 5). In conjunction
541 with the observation of an increased glycogen utilization rate with H-CHO and that
542 fatigue in both treatments coincided with depletion of muscle glycogen to critically
543 low muscle glycogen concentrations, the current findings demonstrate that the

544 availability of muscle glycogen is a primary determinant of fatigue during a repeated
545 exercise bout following short-term recovery.

546

547 Hypoglycemia and subsequent reduction in carbohydrate oxidation late in exercise
548 have been proposed as a major cause of fatigue during prolonged moderate to high-
549 intensity cycling exercise (12). However, it has been consistently demonstrated that
550 fatigue during prolonged moderate to high-intensity running is not associated with
551 hypoglycemia (33, 34, 36). The latter notion was further supported by the current
552 investigation, whereby fatigue was not associated with hypoglycemia in either
553 treatment. Additionally, whilst carbohydrate oxidation during a repeated exercise bout
554 was greater when higher amounts of carbohydrate (≈0.75 $g \cdot kg^{-1} \cdot h^{-1}$) were provided
555 relative to a lower dose (≈0.25 $g \cdot kg^{-1} \cdot h^{-1}$) during recovery, no discernable differences
556 in plasma glucose concentrations or time to exhaustion were shown (15). Indeed,
557 fatigue during prolonged exercise was shown independent of carbohydrate oxidation
558 or avoidance of hypoglycemia (11). Further support for the latter study comes from
559 H-CHO trial in the present investigation, where neither hypoglycemia nor a decline in
560 carbohydrate oxidation was apparent at the cessation of exercise to explain fatigue.
561 Thus, it can be suggested that factors other than hypoglycemia or a decline in
562 carbohydrate oxidation rates limited the capacity for subsequent exercise.

563

564 In conclusion, the ingestion of 1.2 $g \cdot kg^{-1} \cdot h^{-1}$ of carbohydrate during 4 h recovery from
565 an initial exhaustive exercise bout increased muscle glycogen availability prior to a
566 repeated exercise bout when compared with the ingestion of 0.3 $g \cdot kg^{-1} \cdot h^{-1}$. In
567 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.

Acknowledgments

The authors thank the research participants for their time, effort and commitment to the study.

This work is supported by the Saudi Arabian Ministry of Higher Education (s4305). Nutritional supplements have been provided by GlaxoSmithKline.

The authors have no conflicts of interest. The results of the study do not constitute endorsement by ACSM.

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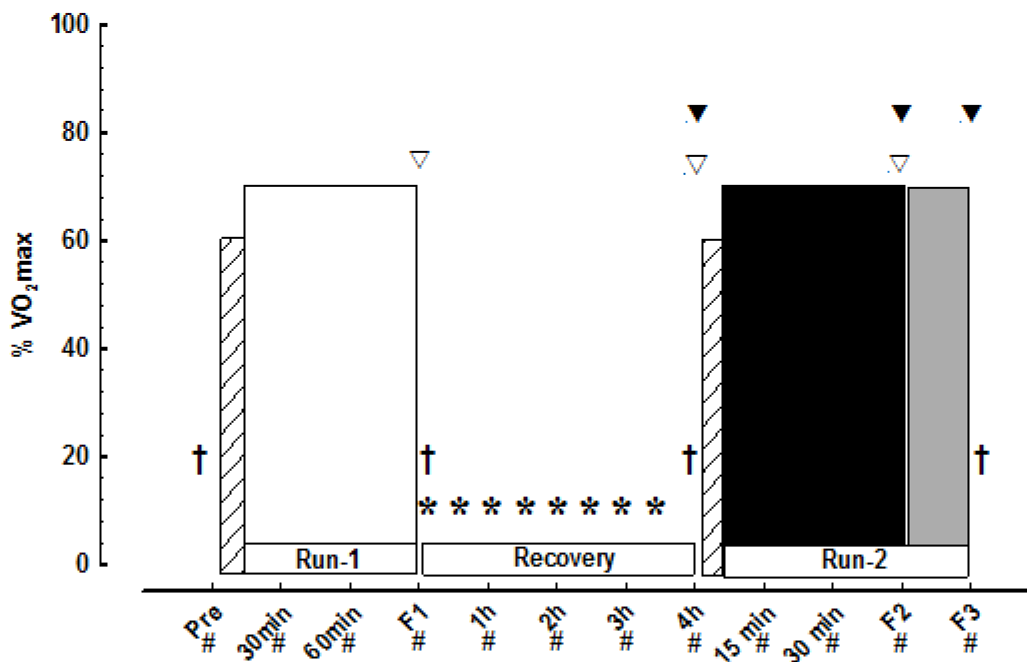
Table 1. Substrate metabolism and respiratory exchange ratio (RER) during Run-1 and Run-2 with L-CHO or H-CHO treatments.

	Run-1					Run-2					
	Pre	30 min	60 min	90 min	F1	15 min	30 min	45 min	F2	60 min	F3
Carbohydrate oxidation (g·min^{-1})											
L-CHO	0.26±0.15	2.20±0.36	2.18±0.37	1.90 ±0.40	1.87±0.72	1.92±0.74	1.99±0.89	2.74±1.04	1.60±0.79		
						*	*		*		#
H-CHO	0.33±0.19	2.59±0.70	2.30±0.59	2.02±0.80	1.98±0.73	2.68±0.68	3.18±1.06	2.81±0.95	2.41±0.46	2.66±0.47	2.41±0.98
Lipid oxidation (g·min^{-1})											
L-CHO	0.06±0.06	0.57±0.20	0.61±0.21	0.76±0.22	0.77±0.36	0.77±0.22	0.71±0.31	0.56±0.31	0.94±0.24		
						*	*		*		#
H-CHO	0.06±0.07	0.44±0.20	0.57±0.23	0.66±0.44	0.70±0.32	0.24±0.17	0.26±0.18	0.40±0.26	0.45±0.16	0.36±0.23	0.50±0.33
RER											
L-CHO	0.87±0.12	0.89± 0.04	0.88±0.04	0.85±0.04	0.85±0.06	0.85±0.05	0.86±0.07	0.90±0.07	0.82±0.05		
						*	*		*		#
H-CHO	0.90±0.11	0.91±0.04	0.89±0.04	0.88±0.08	0.86±0.06	0.96±0.05	0.95±0.04	0.92±0.05	0.91±0.04	0.93±0.02	0.90±0.06

Values are mean ± SD. *, values different between L-CHO and H-CHO ($p < 0.05$); #, values different at absolute fatigue (F2 vs. F3) between L-CHO and H-CHO ($p<0.05$)



694

695 **Figure 1**. A schematic representation of the study protocol †, body mass assessment;
696 *, fluid provision; #, expired gas and blood sample;▽, muscle biopsy during L-CHO;
697 ▼, muscle biopsy during H-CHO; F1, fatigue in Run-1; F2, fatigue in L-CHO; F3,
698 fatigue in H-CHO; dashed columns, warm-up; black column, run time to exhaustion
699 in L-CHO trial; grey column, extended run time to exhaustion with H-CHO treatment
700 during Run-2.

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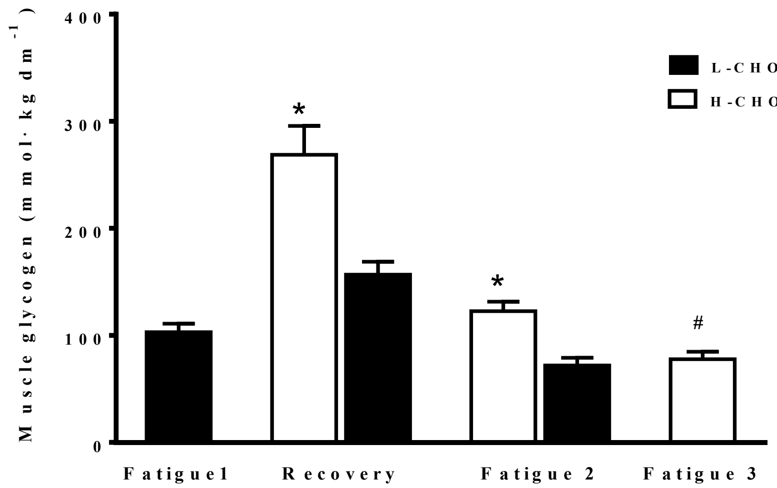

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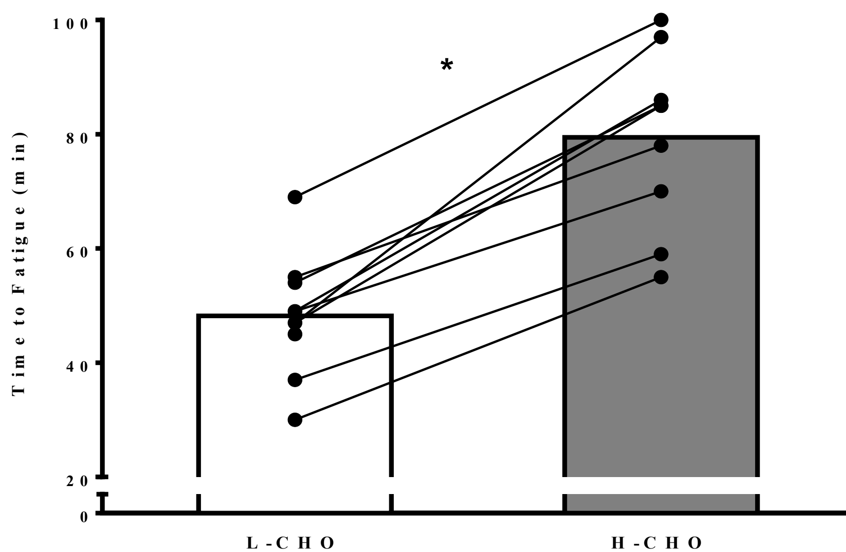

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707 **Figure 2-A**. Muscle glycogen concentrations at the end of Run-1 (F1), at the end of 4

708 h recovery, time to exhaustion with L-CHO treatment (F2) and time to exhaustion

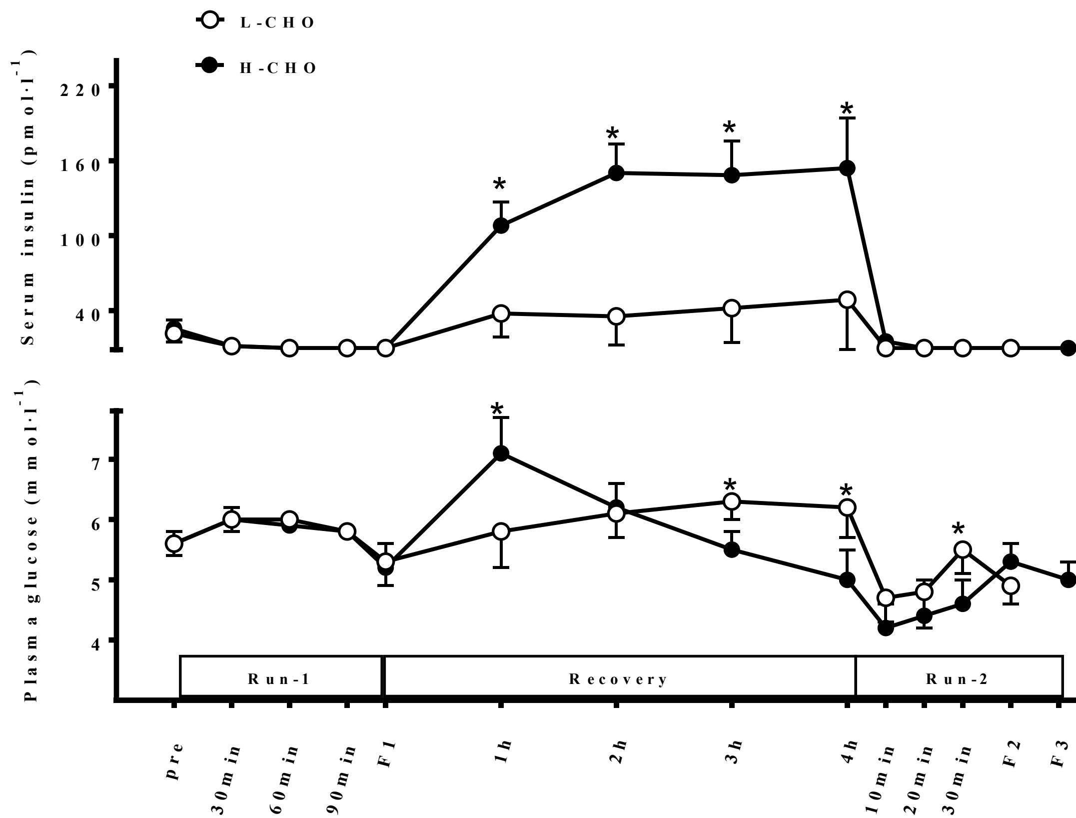
709 with H-CHO treatment (F3). **Figure 2-B**, mean and individual run times to exhaustion

710 following the ingestion of L-CHO or H-CHO during 4 h recovery. Values are means

711 ± CI. *, values different between L-CHO and H-CHO ($p < 0.01$). #, values different

712 between F2 and F3 within the H-CHO treatment ($p < 0.01$).

713



714
715

716 **Figure 3**. Plasma glucose and serum insulin concentrations during Run-1, recovery
717 and Run-2 with L-CHO or H-CHO treatments. Values are mean ± CI. *, values
718 different between L-CHO and H-CHO ($p < 0.05$). F1, time to exhaustion during Run-
719 1; F2, time to exhaustion with L-CHO treatment; F3= time to exhaustion with H-CHO
720 treatment.

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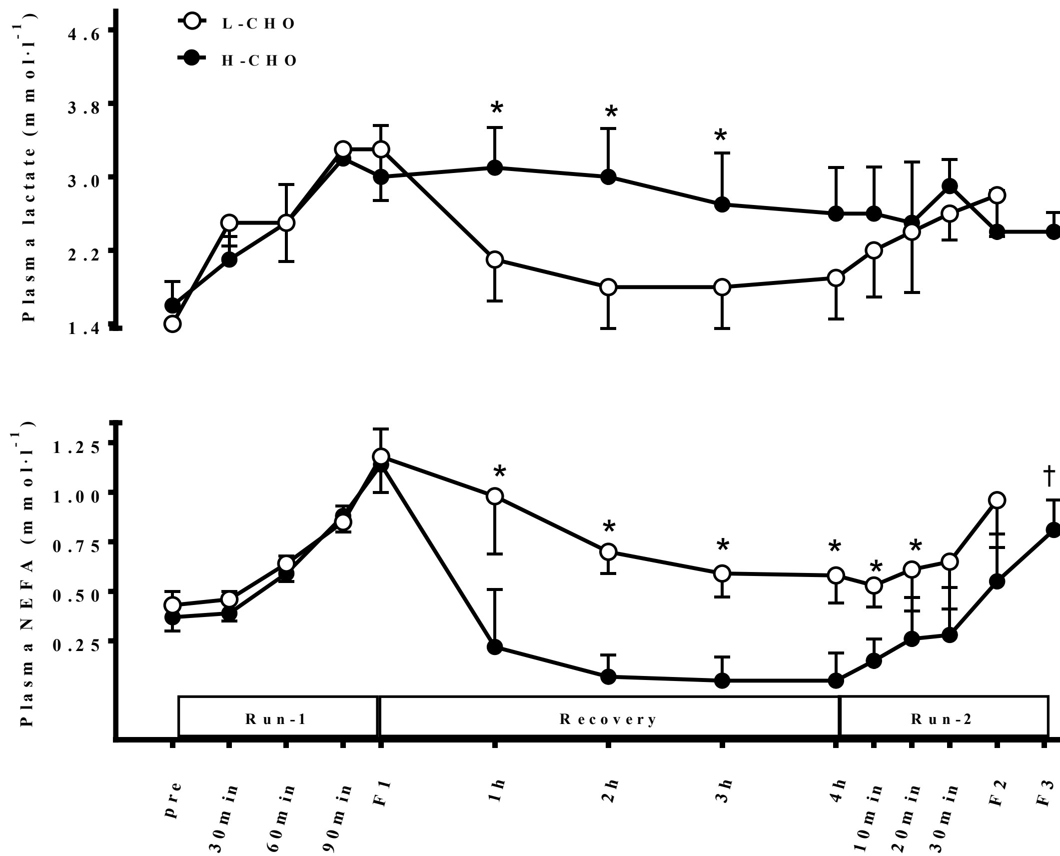

722

723 **Figure 4**. Plasma NEFA and lactate concentrations during Run-1, recovery and Run-2

724 with L-CHO or H-CHO treatments. Values are mean ± CI. *, values different between

725 L-CHO and H-CHO ($p < 0.01$). †, values different from F2 to F3 in H-CHO treatment.

726 F1, time to exhaustion during Run-1; F2, time to exhaustion with L-CHO treatment;

727 F3, time to exhaustion with H-CHO treatment.

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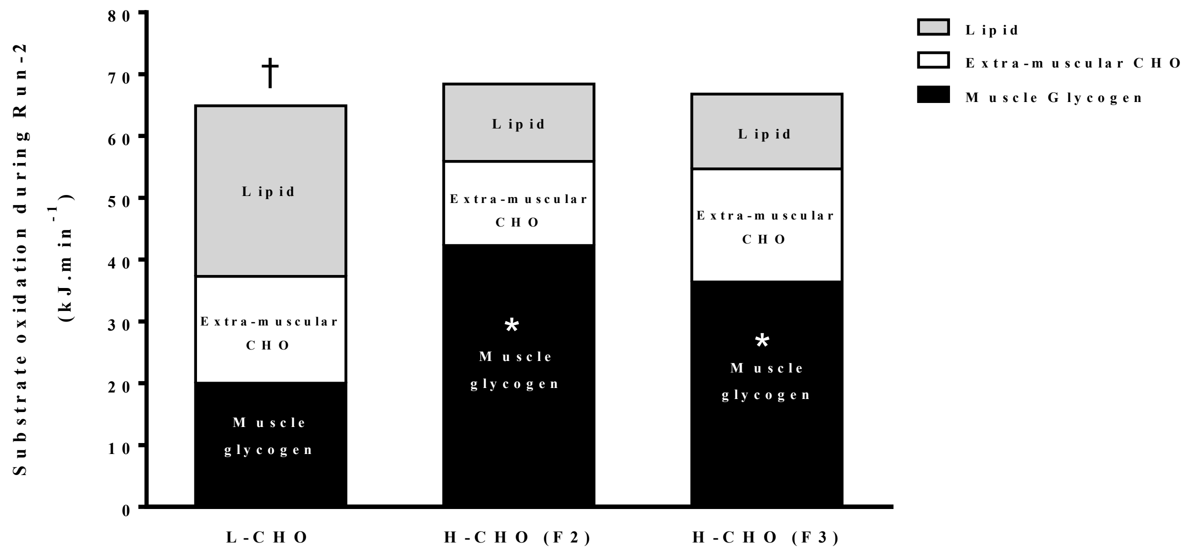

731 **Figure 5**. The contribution of muscle glycogen, extra-muscular carbohydrate (CHO)
732 and lipids to total substrate metabolism (kJ·min^{-1}) during Run-2 with L-CHO or H-
733 CHO treatments. *, Muscle glycogen values different between L-CHO and H-CHO
734 (*p*< 0.05). †= lipid values different between L-CHO and H-CHO treatments. (*p*<
735 0.05), F1, time to exhaustion during Run1; F2, time to exhaustion with L-CHO
736 treatment; F3, time to exhaustion with H-CHO treatment.

737