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1 High rates of fat oxidation are maintained after the sleep low approach despite delayed
2 carbohydrate feeding during exercise

3

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16

17 **Running title:** Delayed carbohydrate feeding

18 **Abstract**

19

20 Training with low carbohydrate availability enhances endurance training adaptations but training
21 volume may be compromised. We explored whole body metabolism and performance with
22 delayed carbohydrate feeding during exercise undertaken following acute sleep low training. We
23 hypothesised this strategy would not suppress fat oxidation and would maintain exercise
24 performance. The study involved 3 experimental trials and included 9 men and 1 woman
25 ($\dot{V}O_{2peak}=58.8\pm 5.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Each trial started in the afternoon with an exhaustive cycling
26 protocol. The following morning 1-h of steady state cycling (SS) was followed by a time trial (TT).
27 Carbohydrates (CHO) were not ingested in recovery from exhaustive exercise or during next day
28 exercise in the Placebo trial (PLA); CHO were not ingested during recovery but were fed (15g every
29 ~15-min) from 30-min into SS and continued during the TT in the delayed feeding trial (DELAY);
30 CHO were provided during recovery (1.2 g/kg/h for 7 hours) and next day exercise (as in DELAY) in
31 a third condition (CHO). Exercise metabolism was assessed using indirect calorimetry and blood
32 sampling. Fat oxidation rates during SS were similar in PLA ($0.83\pm 0.17 \text{ g/min}$) and DELAY
33 ($0.78\pm 0.14 \text{ g/min}$) ($p>0.05$) and higher than CHO ($0.57\pm 0.27 \text{ g/min}$) ($p<0.05$). There were no
34 significant differences in TT performance (49.1 ± 10.7 , 43.4 ± 7.6 , $41.0\pm 7.9 \text{ min}$ in PLA, DELAY and
35 CHO, respectively; $p>0.05$). Delayed carbohydrate feeding could be a strategy to maintain high fat
36 oxidation rates typically associated with exercise undertaken after the sleep low approach to
37 training but the acute performance effects remain inconclusive.

38

39 **Keywords:**

40 Endurance, nutrition, metabolism

41

42 Introduction

43 Recently, the concept of carbohydrate periodization for endurance athletes has emerged whereby
44 dietary carbohydrate intake is tailored to support the varying carbohydrate demands and goals of
45 different types of training and competition (Burke, Hawley, Wong, & Jeukendrup, 2011; Impey et
46 al., 2018). This stems in part from research showing that strategically restricting carbohydrate
47 availability around certain training sessions can enhance metabolic and/or functional adaptations
48 (Hulston et al., 2010; Marquet et al., 2016; Yeo et al., 2008) by augmenting the acute and
49 cumulative adaptive (i.e., molecular) response to exercise (Impey et al., 2018). However,
50 exercising with low carbohydrate availability can decrease exercise capacity (Bergström,
51 Hermansen, Hultman, & Saltin, 1967; Impey et al., 2016) and intensity (Hulston et al., 2010; Yeo et
52 al., 2008). Despite suggestions of increased training efficiency (i.e. comparable molecular signalling
53 response with a lower training volume) (Impey et al., 2016), this strategy might not achieve
54 optimal adaptations due to reduced overall training volume. Maintaining capacity to undertake
55 intense and long duration training sessions whilst in a state of reduced endogenous carbohydrate
56 availability would likely lead to the most favourable adaptations.

57

58 Carbohydrate feeding during exercise can improve exercise performance and capacity (Coggan &
59 Coyle, 1989; Coyle et al., 1983; Stellingwerff & Cox, 2014). However, such a practice is not
60 consistent with the principles of exercising with low carbohydrate availability. Carbohydrate
61 ingestion before and/or during exercise can suppress signalling of key molecular pathways thought
62 to be responsible for skeletal muscle oxidative adaptation (Akerstrom et al., 2006; Civitarese,
63 Hesselink, Russell, Ravussin, & Schrauwen, 2005) and in the long term reduce the response to low
64 glycogen training and endurance training in general (Morton et al., 2009; Van Proeyen, Szlufcik,
65 Nielens, Ramaekers, & Hespel, 2011). For example, Morton et al. observed a blunted increase in

66 succinate dehydrogenase activity and heat shock protein content after 6 weeks of training that
67 included high intensity interval exercise sessions commenced with reduced muscle glycogen stores
68 with carbohydrate intake just before and during the training sessions, as compared to when no
69 carbohydrates were ingested before and during the training sessions. Collectively, it appears that
70 some of the proposed metabolic (adaptive) signals associated with exercise with low muscle
71 glycogen (e.g., elevated lipid metabolism, increased catecholamines) (Philp, Hargreaves, & Baar,
72 2012) are suppressed with carbohydrate provision, further underpinning why carbohydrate
73 feeding during exercise might impede training adaptation.

74

75 Prior studies concerned with using low glycogen availability to optimize training adaptation have
76 not considered the potential for delayed feeding of carbohydrates during exercise. Delaying
77 feeding of carbohydrate until 135-min into a strenuous exercise bout, a time when liver and
78 muscle glycogen content is likely reduced, enhanced exercise capacity but did not alter respiratory
79 exchange ratio (RER) or non-esterified fatty acids (NEFA) concentrations, indicating unaltered
80 metabolic environment (Coggan & Coyle, 1989). However, exercise in this study was commenced
81 without prior manipulation of muscle glycogen, and it is unclear if delaying carbohydrate feeding
82 when exercise is commenced with low glycogen would also maintain elevated lipid metabolism.
83 Carbohydrate feeding immediately after the onset of exercise commenced with reduced muscle
84 glycogen enhances exercise performance (Ali, Yoo, Moss, & Breier, 2016; Widrick et al., 1993).
85 However, the effect of delayed carbohydrate on performance when exercise is commenced with
86 low carbohydrate availability is unknown. Collectively, delaying carbohydrate feeding during
87 exercise commenced under conditions of low glycogen availability has the potential to maintain
88 the metabolic (adaptive signals) but not compromise performance typically associated with
89 exercise with low glycogen, but this remains to be investigated.

90

91 It is an established practice for world class athletes to start training sessions after an overnight fast
92 when liver but not muscle glycogen stores are reduced and start ingesting carbohydrates later in
93 the exercise bout (Froome & Walsh, 2015; Levitch, 2018). Furthermore, a recommended
94 approach for training with low carbohydrate availability is to perform a glycogen depleting session
95 in the afternoon and avoid carbohydrate intake before completing the next training session in the
96 morning without carbohydrate provision (i.e., the 'sleep-low' strategy, low liver and muscle
97 glycogen) (Marquet et al., 2016). We hypothesised that carbohydrate feeding commenced 30
98 minutes after the start exercise would not alter the metabolic environment (e.g. substrate
99 utilisation, plasma NEFA) thought to be critical for training adaptations during exercise performed
100 after the sleep low strategy. We chose to delay carbohydrate feeding by 30 minutes because this
101 strategy has previously been shown to maintain fat oxidation at similar rates to those observed
102 during overnight fasted state moderate intensity exercise (Horowitz, Mora-Rodriguez, Byerley, &
103 Coyle, 1999). Further we hypothesized that delayed feeding would enhance performance, that is
104 typically compromised under conditions of low carbohydrate available when exercise is
105 commenced following the sleep-low strategy.

106

107 Materials and methods

108 **Participants**

109 Ten healthy, endurance-trained participants (9 men, 1 woman) provided written informed consent
110 and completed the study that was approved by a Local Ethics Committee (University of
111 Birmingham [UK] Science, Technology, Engineering and Mathematics Ethical Committee;
112 application number ERN 17-1236). The sample size was selected to be comparable with previous

113 research that has investigated metabolic and performance responses to acute train low
114 interventions (Hearris et al., 2019; Impey et al., 2016, 2015). The main inclusion criteria for taking
115 part in the study was regular participation in endurance-based exercise (e.g., cycling, running or
116 swimming of at least 30-45 min at least 3 times per week, with one bout of >90 min in the prior 4-
117 6 weeks) and having a VO_{2peak} value $\geq 50 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.

118

119 **Experimental design**

120 After preliminary testing, each participant completed a familiarisation trial and three experimental
121 trials each consisting of two exercise sessions; a glycogen-reducing exercise bout in the afternoon
122 of Day 1 and a 1-h steady state exercise bout (SS) immediately followed by a time trial (TT) with a
123 predicted duration of 40-min on the morning of Day 2. The exercise protocol was adapted from a
124 previous study (Currell, Jentjens, & Jeukendrup, 2006). The experimental trials differed in the diet
125 provided for the remainder of the Day 1 (the 7-h refeeding period after the glycogen reducing
126 exercise session) and during the SS and the TT on Day 2. On one occasion participants received
127 carbohydrates at a rate of $1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ during a 7-h re-feeding period and carbohydrates at a
128 rate of 15 g every 15 minutes during the SS of the second exercise bout commencing 30-min after
129 exercise onset (i.e. at 30, 45 and 60-min time points) and $\frac{1}{3}$ and $\frac{2}{3}$ into the TT (CHO). On the
130 other two occasions they received a noncaloric placebo food in the 7-h re-feeding period and
131 carbohydrates during exercise on Day 2 as described above (DELAY) or they were given noncaloric
132 placebo both during re-feeding (Day 1) and during exercise on Day 2 (PLA). The study adopted a
133 double-blinded crossover design in which the order of the trials was randomized using an online
134 research tool (www.randomizer.com). Experimental trials were separated by 6-14 days.

135

136 **Preliminary testing and familiarization trial**

137 Participants performed an incremental test to exhaustion to determine $\dot{V}O_{2peak}$ and W_{max} on a
138 cycle ergometer (Lode, Groningen, Netherlands). The test started at an intensity of 100 W and the
139 workload increased by 30 W every 2 minutes. During the test, gas exchange measurements were
140 made using an automated online gas analysis system (Vyntus, Vyair Medical, IL, US). The highest
141 30-s average of O_2 uptake was considered to represent $\dot{V}O_{2peak}$. W_{max} was calculated as the
142 power output from the last completed stage plus the fraction of the time spent in the next stage
143 multiplied by 30 W.

144

145 Participants were then scheduled for the familiarisation trial that, with the exception of blood
146 sampling, followed the same protocol as the PLA trial (see below).

147

148 **Experimental trials**

149 A schematic overview of the study is depicted in Figure 1.

150

151

INSERT FIGURE 1

152

153 *Day 1.* Prior to entering the laboratory at ~1 pm participants were asked to replicate the diet and
154 activity patterns on the day of the visit and on the day preceding each experimental trial. A high-
155 intensity-interval exercise protocol was run as described previously (Kuipers, Saris, Brouns, Keizer,
156 & ten Bosch, 1989; Wallis et al., 2008). Briefly, after a 5-min warm-up at 50% W_{max} participants
157 cycled at alternating workloads of 90% and 50% W_{max} , respectively, each lasting 2 minutes. Once
158 90 % workload was deemed too demanding for participants despite strong verbal encouragement,
159 90 % intensity was first reduced to 80 % and then to 70 %. When blocks at 70 % W_{max} could not
160 be completed, the exercise session was terminated. Immediately post-exercise participants were

161 given a protein gel which contained 20 g protein (WHEY 20, Science in Sport, Nelson, UK) and the
162 7-h feeding period (explained below) was initiated. The protein gel was provided to minimize
163 hunger in order to further support the blinding of the study. This type of glycogen reduction and
164 repletion protocol has previously been shown (Dent, Stocks, Ogden, Zemp, & Philp, 2017) to result
165 in muscle glycogen concentrations of $194.6 \pm 52.3 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{dw}$ and $475.3 \pm 43.9 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{dw}$ (or
166 $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{dw}$) the following morning after PLA and CHO, respectively.

167

168 *Day 2.* The next morning (i.e. ~7 am) participants reported to the laboratory after an overnight
169 fast. Upon arrival an indwelling cannula was placed in an antecubital arm vein and a baseline
170 blood sample taken. Immediately after participants received a further identical protein gel and
171 rested for 45 minutes, after which a second blood sample was obtained and the SS part of exercise
172 at 50% W_{max} commenced. Ingestion of 20 g of protein 45-min before exercise has previously
173 been shown not to influence NEFA availability and fat oxidation rates as compared to a fasted
174 condition (Impey et al., 2015). During the SS, $\dot{V}O_2$ and $\dot{V}CO_2$ were quantified every 15 minutes (i.e.,
175 15, 30, 45 and 60 min) by participants breathing into the mouthpiece for 3 minutes, while blood
176 samples were collected at 30 and 60-min time points. Immediately on completion of the SS the TT
177 started. Participants had to perform a certain amount of work (equal to ~40 min of cycling at 65%
178 W_{max}) as quickly as possible as described in detail by Currell et al. (2006), who reported the test to
179 have a coefficient of variability of 4.5%.

180

181 The amount of work for each participant was calculated according to the following equation:

182

183 Total amount of work = $0.65 W_{\text{max}} \times 2,400 \text{ J}$

184

185 The ergometer was set in the linear mode and the linear factor calculated according to the
186 formula:

187

$$188 \quad L = W / (\text{RPM})^2$$

189

190 Where L is a linear factor, W is predicted power and RPM is the cycling cadence. RPM was set to
191 80, whereas W represented 65% Wmax

192

193 Furthermore, experimental recommendations for performance testing were followed (Currell &
194 Jeukendrup, 2008). Following the TT, a final blood sample was collected.

195

196 **Nutritional manipulation**

197 The nutritional manipulation on Day 1 after the glycogen reducing exercise involved receiving 1.2 g
198 $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ of a 2:1 maltodextrin and fructose (MyProtein, Cheshire, UK) mixture (CHO) or the same
199 volume of placebo (PLA and DELAY) for 7 hours. The intervention was delivered every 30-min (0.6
200 $\text{g} \cdot \text{kg}^{-1}$ at each time point) by incorporation of both sugars in the above ratio into a noncaloric
201 beverage (Robinsons, Herts, UK) and a sugar free jelly (Hartley's, Leeds, UK). Apart from the food
202 provided, participants were not allowed to consume any other food. The second part of the
203 nutritional manipulation involved intake during the SS and the TT exercise on Day 2. During the SS,
204 participants received 200 ml of a non-caloric beverage at 15-min time point in all 3 conditions. In
205 PLA participants kept receiving the same volume at 30, 45 and 60-min time points and at $\frac{1}{3}$ and
206 $\frac{2}{3}$ of the completed amount of work during the TT, whereas in DELAY and CHO, 15g of
207 maltodextrin (MyProtein, Cheshire, UK) was added to the beverage at these time points (7%
208 concentration). After each trial, a questionnaire was given to participants asking them to state the

209 condition they believed they had undertaken. Less than 50% of subjects correctly guessed the
210 condition, showing that blinding was successful.

211

212 **Blood analyses**

213 Venous blood samples (~6 mL) were collected into EDTA tubes, stored on ice and then centrifuged
214 at 4°C and 1006 × g for 15 minutes. Aliquots of plasma were then stored at -70°C and later
215 analysed for glucose (Glucose Oxidase kit; Instrumentation Laboratories, Cheshire, UK), NEFA
216 (Randox, London, UK) and lactate (Randox, London, UK) using an ILAB 650 Clinical Chemistry
217 Analyzer (Instrumentation Laboratory, Warrington, UK) and insulin using a commercially available
218 ultrasensitive ELISA kit (Merckodia AB, Uppsala, Sweden). Area under the curve (AUC) was
219 calculated between time points 'baseline' and 60-min of SS.

220

221 **Gas exchange measurements**

222 Fat and carbohydrate oxidation rates were calculated using stoichiometric equations of
223 Jeukendrup and Wallis (2005) assuming protein oxidation to be negligible.

224

225 **Heart rate and ratings of perceived exertion**

226

227 Heart rate (HR) values were obtained at 15-min intervals during the SS. Simultaneously every 15-
228 min participants were asked to report the rate of perceived exertion (RPE) using 6-20 scale (Borg,
229 1982).

230

231 **Statistics**

232

233 Data were initially tested for sphericity using Mauchly's test. Then, a two-way ANOVA for
234 repeated-measures was used to compare differences in substrate utilization and blood
235 metabolites. When necessary, analyses were adjusted using the Greenhouse–Geisser correction. A
236 one-way ANOVA was used to compare AUC and time for the TT completions. Where significant
237 effects were observed by ANOVA, post-hoc pair-wise comparisons were made with paired t-tests
238 with the Tukey test applied to account for multiple comparisons. Effect sizes (ES) for TT
239 performance were calculated using Hedge's *g*, where 0.2-0.5 represented a small, 0.5-0.8
240 moderate and >0.8 a large effect. All values are presented as mean \pm SD. Statistical significance
241 was set at $p < 0.05$. Statistics were performed using SPSS (Version 21; SPSS Inc., Chicago, IL, US)
242 and Prism (Version 8; GraphPad Software, San Diego, CA, US).

243

244 Results

245 **Participants' characteristics**

246 The participants' characteristics were as follows: mean age: 27 ± 5 years, body mass: 67.7 ± 5 kg,
247 height: 176 ± 7 cm, maximal oxygen uptake ($\dot{V}O_{2peak}$): 4.0 ± 0.4 L \cdot min⁻¹ (58.8 ± 4.9 mL \cdot kg⁻¹ \cdot min⁻¹),
248 and maximal cycle ergometer power output (W_{max}): 351 ± 46 W (5.2 ± 0.8 W \cdot kg⁻¹).

249

250 **Glycogen-reducing session (Day 1)**

251 Time to complete the glycogen reducing sessions in in CHO, PLA and DELAY, respectively, were 124
252 ± 31 ; 126 ± 35 and 123 ± 42 minutes, without any statistically significant differences between the
253 trials ($p = 0.920$). Participants completed 1701 ± 429 , 1750 ± 512 and 1693 ± 595 kJ of mechanical
254 work during the glycogen reducing sessions in CHO, PLA and DELAY, respectively, without any
255 statistically significant differences between the trials ($p = 0.966$). Neither were there any

256 differences in the number of completed stages at 90, 80 and 70 % W_{max} between all three
257 conditions ($p = 0.920$).

258

259 **Fat and carbohydrate oxidation rates, $\dot{V}O_2$, RER, RPE and HR during steady state exercise (Day 2)**

260

261 Fat and carbohydrate oxidation rates are presented in Figure 2 whereas % $\dot{V}O_{2peak}$, HR and RPE
262 during the SS exercise bout are presented in Table 1 and grouped into time frames before (0-30
263 min) or after (30-60 min) a time point at which in DELAY and CHO carbohydrates started to be
264 ingested.

265

266

INSERT FIGURE 2

267

268 As shown in Figure 2, carbohydrate oxidation was lower and fat oxidation higher throughout the
269 SS in PLA ($p = 0.014$ and $p = 0.012$; for carbohydrate and fat oxidation, respectively) and DELAY (p
270 $= 0.041$ and $p = 0.045$; for carbohydrate and fat oxidation, respectively) as compared with CHO,
271 while there was no difference between PLA and DELAY ($p = 0.87$ and $p = 0.805$; for carbohydrate
272 and fat oxidation, respectively). In all conditions, carbohydrate oxidation decreased, while fat
273 oxidation increased over time ($p < 0.001$). Furthermore, there was no significant difference in RER
274 values between DELAY (0.82 ± 0.03) and PLA (0.81 ± 0.04) ($p = 0.915$), while both differed as
275 compared to CHO (0.87 ± 0.06) ($p = 0.039$ and $p = 0.016$ for DELAY and PLA, respectively).

276

277

INSERT TABLE 1

278

279 As shown in Table 1, there were no differences in % $\dot{V}O_{2peak}$ between conditions ($p = 0.022$), but it
280 increased to a similar extent in all conditions over time ($p = 0.025$). Also, there was no effect of
281 time ($p = 0.552$) or condition ($p = 0.338$) for HR. RPE increased over time in all 3 conditions ($p =$
282 0.006). It was significantly higher in DELAY (14 ± 3) when compared to CHO (13 ± 2 ; $p = 0.036$), and
283 tended to be higher in PLA (14 ± 3) than CHO ($p = 0.055$), whilst being similar between and PLA and
284 DELAY (0.975).

285

286 **Plasma, NEFA, insulin, glucose and lactate during exercise (Day 2)**

287 Results for NEFA, Insulin, glucose and lactate are presented in Figure 3.

288

289

INSERT FIGURE 3

290

291 NEFA concentrations (Figure 3a) were lower at the baseline in CHO ($0.9 \pm 0.5 \text{ mmol} \cdot \text{L}^{-1}$) as
292 compared to PLA ($1.5 \pm 0.4 \text{ mmol} \cdot \text{L}^{-1}$; $p < 0.001$) and DELAY ($1.6 \pm 0.8 \text{ mmol} \cdot \text{L}^{-1}$; $p < 0.001$). NEFA
293 concentrations dropped from the baseline to 0-min time point in all conditions ($p < 0.05$) and
294 there were no differences between conditions in absolute concentrations ($p > 0.05$). After 30-min
295 of SS, NEFA concentrations increased in all conditions ($p < 0.05$). However, the increase was less
296 pronounced in CHO in comparison to PLA and DELAY, where values were significantly higher at this
297 time point at 0.7 ± 0.5 , 1.2 ± 0.6 and $1.1 \pm 0.7 \text{ mmol} \cdot \text{L}^{-1}$ in CHO, PLA and DELAY, respectively ($p <$
298 0.05). Concentrations did not further change neither in PLA and CHO ($p > 0.05$), whereas
299 insignificantly dropped to $0.8 \pm 0.5 \text{ mmol} \cdot \text{L}^{-1}$ in DELAY from 30 to 60-min time point ($p = 0.165$) so
300 that at 60-min time point DELAY and CHO values were not statistically significantly different ($p =$
301 0.994). AUC for NEFA was significantly lower in CHO as compared with PLA ($p = 0.007$) and DELAY
302 ($p = 0.042$), without being different between DELAY and PLA (0.678).

303

304

305 Insulin concentrations (Figure 3b) did not differ at the baseline ($p > 0.05$) and were only marginally
306 increased just before the SS ($p > 0.05$). At the 30-min time point insulin concentrations dropped
307 similarly in all conditions as compared to 0-min, although the decrease was only significant in PLA
308 ($-4.1 \pm 2.3 \text{ mU} \cdot \text{L}^{-1}$; $p < 0.001$) and CHO ($-3.5 \pm 3 \text{ mU} \cdot \text{L}^{-1}$; $p = 0.003$) and not in DELAY ($-2.5 \pm 2 \text{ mU} \cdot$
309 L^{-1} ; $p = 0.178$) condition. Insulin concentrations did not change significantly between 30-min and
310 60-min in any condition ($p > 0.05$). Nonetheless, they were significantly higher in DELAY ($+ 3.6 \pm$
311 $3.5 \text{ mU} \cdot \text{L}^{-1}$; $p = 0.003$) and CHO ($+ 4.7 \pm 3.0$; $\text{mU} \cdot \text{L}^{-1}$ $p < 0.001$) as compared with PLA, whereas
312 there was no difference between DELAY and CHO ($p > 0.999$) at 60-min. AUC for Insulin was
313 significantly higher in CHO as compared with PLA ($p = 0.034$), whereas there was no difference
314 between CHO and DELAY ($p = 0.194$) or PLA and DELAY ($p = 0.619$).

315

316 At baseline, before the SS (0-min) and at mid-point of the SS (30-min) concentrations of glucose
317 (Figure 3c) were not different between conditions ($p > 0.05$). Concentrations remained stable for
318 the rest of the SS in PLA ($p > 0.05$), whereas glucose concentration increased by $1.3 \pm 0.6 \text{ mmol} \cdot \text{L}^{-1}$
319 1 in DELAY from 30-min to 60-min time point ($p < 0.001$) and by $0.7 \pm 0.6 \text{ mmol} \cdot \text{L}^{-1}$ in CHO ($p =$
320 0.009). Concentrations did not change at the end of the TT in CHO and DELAY, whereas
321 concentrations significantly decreased ($-0.8 \pm 0.3 \text{ mmol} \cdot \text{L}^{-1}$) at the end of the TT in PLA ($p <$
322 0.001). Glucose concentrations were higher in CHO (5.4 ± 0.8 and $5.1 \pm 1.3 \text{ mmol} \cdot \text{L}^{-1}$) and DELAY
323 (5.6 ± 0.7 and $5.2 \pm 0.6 \text{ mmol} \cdot \text{L}^{-1}$) as compared to PLA (4.2 ± 0.6 and $3.3 \pm 0.6 \text{ mmol} \cdot \text{L}^{-1}$) at 60-
324 min and post TT time points ($p < 0.05$) with no difference between CHO and DELAY ($p > 0.999$)
325 conditions. AUC for glucose was significantly higher in CHO as compared with PLA ($p = 0.006$),

326 whereas there was no difference between CHO and DELAY ($p = 0.189$) or PLA and DELAY ($p =$
327 0.228).

328

329

330 Lactate concentrations (Figure 3d) remained constant during the SS and only significantly
331 increased post TT in all three conditions ($p < 0.05$) with only significant difference between CHO
332 and PLA ($p < 0.001$), without differences between PLA and DELAY ($p = 0.127$) or DELAY and CHO (p
333 $= 0.774$). AUC for lactate was significantly higher in CHO as compared with PLA ($p = 0.029$) and
334 DELAY ($p = 0.019$), whereas there was no difference between PLA and DELAY ($p = 0.974$).

335

336 **TT performance**

337

338 Only 9 participants successfully finished all TTs, while one participant could not finish the TT in PLA
339 condition reporting blurred vision and light-headedness. Later analysis showed this participant
340 developed hypoglycaemia with plasma glucose concentrations of $2.7 \text{ mmol} \cdot \text{L}^{-1}$ at the point of
341 fatigue. This participant's data was not included in the analysis of performance responses.

342 Participants completed the TT in 41.0 ± 7.9 , 49.1 ± 10.7 and 43.4 ± 7.6 (minutes in CHO, PLA and
343 DELAY conditions, respectively, with no statistically significant differences between the trials ($p =$
344 0.094). ES comparisons for DELAY vs. PLA, CHO vs. PLA and CHO vs. DELAY were 0.57 (moderate),
345 0.8 (large) and 0.3 (small). TT results with mean values and individual data points are presented in
346 Figure 4.

347

348

INSERT FIGURE 4

349

350 Discussion

351

352 The main aim of this study was to explore how delayed carbohydrate feeding during subsequent
353 exercise, when following the sleep-low approach to training with low carbohydrate availability,
354 affected whole-body metabolism. A primary finding was that delayed carbohydrate feeding did
355 not compromise the high fat oxidation rates typically observed during exercise commenced with
356 low carbohydrate availability. Secondly, we explored how exercise performance was affected by
357 delayed carbohydrate feeding during exercise following an acute sleep-low intervention. The
358 present study did not show any significant differences in TT performance. However, this
359 observation must be interpreted with caution as the study was underpowered to detect significant
360 performance differences, and one participant's data was excluded due to failure to complete the
361 performance test in the PLA condition.

362

363 Overall fat oxidation rates during exercise on Day 2 were higher in both sleep-low conditions (PLA
364 and DELAY) as compared to when carbohydrates were provided in recovery (CHO). Furthermore,
365 during exercise on Day 2 delayed carbohydrate feeding in DELAY did not prevent an increase in
366 rates of fat oxidation so that in PLA and DELAY they remained comparable. This provides further
367 support for a concept that low carbohydrate availability and particularly low muscle glycogen
368 determine fat oxidation rates during exercise (Arkinstall et al., 2004). It is also important to
369 recognise that apart from different carbohydrate availability, overall energy availability was
370 different as well (i.e., lower in sleep-low conditions). The elevated fat oxidation in DELAY occurred
371 despite NEFA concentrations being reduced to concentrations similar to those observed in CHO

372 and thus below those seen in PLA. The reduction in NEFA concentrations most likely occurred as a
373 result of the reduction of adipose tissue lipolysis because of insulin (Campbell, Carlson, Hill, &
374 Nurjhan, 1992). Our results therefore show that delayed feeding in DELAY caused a divergence
375 between fat oxidation rates and NEFA availability. This contrasts some previous work showing that
376 a suppression of NEFA availability is associated with reduced fat oxidation rates (Horowitz, Mora-
377 Rodriguez, Byerley, & Coyle, 1997). While speculative, intramuscular triacylglycerol (IMTG)
378 utilization could have been increased (van Loon et al., 2005; Watt et al., 2004) and become an
379 important source of fatty acids in DELAY partially replacing plasma borne NEFA.

380

381 From the perspective of training adaptations, the significance of the divergence in fat oxidation
382 and NEFA availability during DELAY is unclear. Delayed carbohydrate feeding in DELAY increased
383 fat oxidation rates as compared to CHO, but suppressed plasma NEFA availability as compared with
384 PLA. As implied in the present study fat oxidation during exercise not only relies on plasma NEFA,
385 but also on IMTG. Thus a high flux through lipid metabolism pathways could be sustained by IMTG
386 utilization which could also act as a signal for molecular adaptations (Meex et al., 2015; Philp et
387 al., 2012). There are multiple proposed mechanisms on why training with reduced muscle
388 glycogen content might promote desirable molecular signalling (e.g. AMPK), which include
389 elevated plasma NEFA concentrations and glycogen depletion (Philp et al., 2012). It has been
390 implied that increased NEFA could directly cause augmentation of molecular signalling (e.g. PPAR
391 and p38MAPK) that would in the long term lead to favourable adaptations (Philp et al., 2013;
392 Zbinden-Foncea, Van Loon, Raymackers, Francaux, & Deldicque, 2013). Even though NEFA
393 concentrations declined with delayed carbohydrate feeding overall exposure as assessed by NEFA
394 AUC was similar between PLA and DELAY, thus it could be speculated that the overall NEFA
395 stimulus is maintained with DELAY. Further research is required to better understand whether the

17

396 crucial signal is NEFA availability and/or high muscle fat utilisation per se (i.e., high fat oxidation
397 rates) that are most important for promoting training adaptations when exercising under
398 conditions of low muscle glycogen.

399

400 Undertaking exercise sessions in a muscle glycogen depleted state compromises ability to exercise
401 at high intensities (Hulston et al., 2010; Yeo et al., 2008). We delayed the feeding in conditions of
402 low carbohydrate availability in an attempt to maximise the lipid metabolic response to exercise,
403 however in this context we were unable to discriminate performance differences between any of
404 the study conditions. We based our protocol on a previous study showing a good reliability of the
405 TT performance in the state of low carbohydrate availability (Currell et al., 2006). However, this TT
406 performance test has not been investigated in terms of sensitivity, i.e. whether it is able to detect
407 small, but meaningful changes in performance. Poor sensitivity of the test could thus be a reason
408 for lack of performance differences observed in the present study. Despite familiarization, a large
409 variability in TT performance was observed in response to the experimental conditions between
410 participants which undoubtedly also contributed to the failure to reveal clear performance
411 differences. Another explanation for lack of significant findings might be a small sample size.
412 Indeed, a post-hoc power calculation showed that there was a 60%, 35% and 13% chance of
413 detecting a significant difference between CHO vs. PLA, DELAY vs. PLA and CHO vs DELAY,
414 respectively. Nonetheless, the direction of the change in exercise performance and the effect sizes
415 observed were in line with what might have been predicted thus indicating a potential for rescuing
416 of performance in DELAY. This would be in line with a recent study demonstrating a better
417 capacity to sustain high intensity efforts with higher muscle glycogen content at the start of the
418 exercise (Hearris et al., 2019).

419

420 Although there were no clear performance differences, the plasma glucose concentration data is
421 potentially revealing. Maintenance of circulating glucose concentrations during exercise is often
422 considered one of the key mechanisms underpinning the ergogenic effect of carbohydrate
423 feeding, particularly in studies of exercise capacity (Christensen & Hansen, 1939; Coggan & Coyle,
424 1989; Coyle, Coggan, Hemmert, & Ivy, 1986). Our results showed diminishing plasma glucose
425 concentrations in the PLA condition, and indeed one participant failed to complete the TT in PLA
426 which could be attributed to hypoglycaemia (plasma glucose $2.7 \text{ mmol} \cdot \text{L}^{-1}$). In contrast, plasma
427 glucose concentrations were maintained in DELAY at comparable levels to those seen in CHO. This
428 raises the possibility that had exercise capacity been assessed, and not TT performance,
429 endurance could have been increased more consistently with delayed feeding. This notion is a
430 speculation, but it is noteworthy that the participant unable to finish the trial in PLA was able to
431 complete the other trials without difficulty. While further research is required, delayed feeding
432 could potentially enable athletes to increase the duration of the training sessions undertaken in
433 glycogen depleted state. This could be beneficial for athletes seeking to increase total duration of
434 training at lower intensities, or for those wishing to maximise the metabolic benefits of training
435 under conditions with elevated fat oxidation rates. It has to be acknowledged that elite athletes
436 train in excess of 20 hours a week with training sessions lasting up-to 6 hours (Jeukendrup, Craig,
437 & Hawley, 2000) and thus limited duration of training with the conventional sleep low approach
438 without delayed carbohydrate intake might not be desirable.

439

440 In summary, the present study demonstrates that delayed feeding with a moderate dose of
441 carbohydrates did not prevent an increase in fat oxidation rates during exercise typically observed
442 with training under conditions of low carbohydrate availability. Delayed carbohydrate feeding
443 during exercise could therefore be an effective way of undertaking endurance training in a state of

444 muscle glycogen depletion with an aim to achieve high fat oxidation rates and to prevent
445 hypoglycaemia response with avoidance of carbohydrates in recovery and training bouts.
446 Nonetheless, further research is required to understand muscle metabolic and molecular
447 responses to such an intervention, its potential to impact exercise capacity or performance and
448 ultimately the impact on long-term training adaptations.

449

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