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

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Running title: Delayed carbohydrate feeding

Abstract

Training with low carbohydrate availability enhances endurance training adaptations but training volume may be compromised. We explored whole body metabolism and performance with delayed carbohydrate feeding during exercise undertaken following acute sleep low training. We hypothesised this strategy would not suppress fat oxidation and would maintain exercise performance. The study involved 3 experimental trials and included 9 men and 1 woman ($\dot{V}O_2$peak=58.8±5.5 mL · kg^{-1} · min^{-1}). Each trial started in the afternoon with an exhaustive cycling protocol. The following morning 1-h of steady state cycling (SS) was followed by a time trial (TT). Carbohydrates (CHO) were not ingested in recovery from exhaustive exercise or during next day exercise in the Placebo trial (PLA); CHO were not ingested during recovery but were fed (15g every ~15-min) from 30-min into SS and continued during the TT in the delayed feeding trial (DELAY); CHO were provided during recovery (1.2 g/kg/h for 7 hours) and next day exercise (as in DELAY) in a third condition (CHO). Exercise metabolism was assessed using indirect calorimetry and blood sampling. Fat oxidation rates during SS were similar in PLA (0.83±0.17 g/min) and DELAY (0.78±0.14 g/min) ($p>0.05$) and higher than CHO (0.57±0.27 g/min) ($p<0.05$). There were no significant differences in TT performance (49.1±10.7, 43.4±7.6, 41.0±7.9 min in PLA, DELAY and CHO, respectively; $p>0.05$). Delayed carbohydrate feeding could be a strategy to maintain high fat oxidation rates typically associated with exercise undertaken after the sleep low approach to training but the acute performance effects remain inconclusive.

Keywords:

Endurance, nutrition, metabolism

Introduction

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

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

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

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

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

Materials and methods

Participants

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

research that has investigated metabolic and performance responses to acute train low interventions (Hearris et al., 2019; Impey et al., 2016, 2015). The main inclusion criteria for taking part in the study was regular participation in endurance-based exercise (e.g., cycling, running or swimming of at least 30-45 min at least 3 times per week, with one bout of >90 min in the prior 4-6 weeks) and having a VO_2peak value ≥50 mL · kg^{-1} · min^{-1}.

Experimental design

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

Preliminary testing and familiarization trial

137 Participants performed an incremental test to exhaustion to determine $\dot{V}O_2$peak and Wmax 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, Vyaire Medical, IL, US). The highest
141 30-s average of O_2 uptake was considered to represent $\dot{V}O_2$peak. Wmax 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% Wmax participants
157 cycled at alternating workloads of 90% and 50% Wmax, 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 % Wmax could not
160 be completed, the exercise session was terminated. Immediately post-exercise participants were

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

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

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

Total amount of work = 0.65 Wmax × 2,400 J

185 The ergometer was set in the linear mode and the linear factor calculated according to the
186 formula:
187
188 $L = W / (RPM)^2$
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 $\cdot kg^{-1} \cdot h^{-1}$ 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 $g \cdot kg^{-1}$ 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 $^1/_3$ and
206 $^2/_3$ 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

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

Blood analyses

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

Gas exchange measurements

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

Heart rate and ratings of perceived exertion

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

Statistics

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 ± 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 ($\dot{V}O_2$peak): 4.0 ± 0.4 L· min^{-1} (58.8 ± 4.9 mL · kg^{-1} · min^{-}
248 ^{1}), and maximal cycle ergometer power output (Wmax): 351 ± 46 W (5.2 ± 0.8 W · 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

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

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

Fat and carbohydrate oxidation rates are presented in Figure 2 whereas % $\dot{V}O_2$peak, HR and RPE during the SS exercise bout are presented in Table 1 and grouped into time frames before (0-30 min) or after (30-60 min) a time point at which in DELAY and CHO carbohydrates started to be ingested.

INSERT FIGURE 2

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

INSERT TABLE 1

279 As shown in Table 1, there were no differences in %$\dot{V}O_2$peak 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 ± 0.5 mmol · L^{-1}) as
292 compared to PLA (1.5 ± 0.4 mmol · L^{-1}; $p < 0.001$) and DELAY (1.6 ± 0.8 mmol · 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 ± 0.7 mmol · 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 ± 0.5 mmol · 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).

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

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

Discussion

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

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

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 mmol · 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

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

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