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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

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 ≥50 mL · kg^{-1} · 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 g · kg^{-1} · 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 $^1/_3$ and $^2/_3$ 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**

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

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

Experimental trials

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

INSERT FIGURE 1

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

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

Results

Participants' characteristics

The participants' characteristics were as follows: mean age: 27 ± 5 years, body mass: 67.7 ± 5 kg, 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^{-1}), and maximal cycle ergometer power output (Wmax): 351 ± 46 W (5.2 ± 0.8 W · kg^{-1}).

Glycogen-reducing session (Day 1)

Time to complete the glycogen reducing sessions in in CHO, PLA and DELAY, respectively, were 124 ± 31; 126 ± 35 and 123 ± 42 minutes, without any statistically significant differences between the trials ($p = 0.920$). Participants completed 1701 ± 429, 1750 ± 512 and 1693 ± 595 kJ of mechanical work during the glycogen reducing sessions in CHO, PLA and DELAY, respectively, without any 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

As shown in Table 1, there were no differences in %$\dot{V}O_2$peak between conditions (p = 0.022), but it increased to a similar extent in all conditions over time (p = 0.025). Also, there was no effect of time (p = 0.552) or condition (p = 0.338) for HR. RPE increased over time in all 3 conditions (p = 0.006). It was significantly higher in DELAY (14±3) when compared to CHO (13±2; p = 0.036), and tended to be higher in PLA (14±3) than CHO (p = 0.055), whilst being similar between and PLA and DELAY (0.975).

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

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

INSERT FIGURE 3

NEFA concentrations (Figure 3a) were lower at the baseline in CHO (0.9 ± 0.5 mmol · L^{-1}) as 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 concentrations dropped from the baseline to 0-min time point in all conditions (p < 0.05) and there were no differences between conditions in absolute concentrations (p > 0.05). After 30-min of SS, NEFA concentrations increased in all conditions (p < 0.05). However, the increase was less pronounced in CHO in comparison to PLA and DELAY, where values were significantly higher at this 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 < 0.05). Concentrations did not further change neither in PLA and CHO (p > 0.05), whereas insignificantly dropped to 0.8 ± 0.5 mmol · L^{-1} in DELAY from 30 to 60-min time point (p = 0.165) so that at 60-min time point DELAY and CHO values were not statistically significantly different (p = 0.994). AUC for NEFA was significantly lower in CHO as compared with PLA (p = 0.007) and DELAY (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 $\cdot L^{-1}$; $p < 0.001$) and CHO (-3.5 ± 3 mU $\cdot L^{-1}$; $p = 0.003$) and not in DELAY (-2.5 ± 2 mU $\cdot L^{-1}$; $p = 0.178$) 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 ($+ 3.6 \pm 3.5$ mU $\cdot L^{-1}$; $p = 0.003$) and CHO ($+ 4.7 \pm 3.0$; mU $\cdot L^{-1}$ $p < 0.001$) 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 $\cdot L^{-1}$ in DELAY from 30-min to 60-min time point ($p < 0.001$) and by 0.7 ± 0.6 mmol $\cdot L^{-1}$ 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 $\cdot L^{-1}$) 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 $\cdot L^{-1}$) and DELAY (5.6 ± 0.7 and 5.2 ± 0.6 mmol $\cdot L^{-1}$) as compared to PLA (4.2 ± 0.6 and 3.3 ± 0.6 mmol $\cdot L^{-1}$) 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 ($p = 0.006$),

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

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

TT performance

Only 9 participants successfully finished all TTs, while one participant could not finish the TT in PLA condition reporting blurred vision and light-headedness. Later analysis showed this participant developed hypoglycaemia with plasma glucose concentrations of 2.7 mmol · L^{-1} at the point of fatigue. This participant's data was not included in the analysis of performance responses. Participants completed the TT in 41.0 ± 7.9, 49.1 ± 10.7 and 43.4 ± 7.6 (minutes in CHO, PLA and DELAY conditions, respectively, with no statistically significant differences between the trials (p = 0.094). ES comparisons for DELAY vs. PLA, CHO vs. PLA and CHO vs. DELAY were 0.57 (moderate), 0.8 (large) and 0.3 (small). TT results with mean values and individual data points are presented in Figure 4.

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

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

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

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

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

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

In summary, the present study demonstrates that delayed feeding with a moderate dose of carbohydrates did not prevent an increase in fat oxidation rates during exercise typically observed with training under conditions of low carbohydrate availability. Delayed carbohydrate feeding 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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