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# **Periodised Carbohydrate Intake Does Not Affect Substrate Oxidation but May Contribute to Endurance Capacity**

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## Abstract

The aim of this study was to investigate whether periodising carbohydrate intake around specific training sessions will enhance endurance training adaptations.

Seventeen healthy recreationally endurance-trained males ( $n = 5$ ) and females ( $n = 12$ ) ( $27.5 \pm 5.4$  years) participated in a four-week training intervention. Participants were divided into two groups: FASTED (stayed fasted between evening high-intensity interval training session and low-intensity training session in the following morning) and FED (no restriction in food intake). Pre- and post-testing included peak oxygen uptake ( $VO_{2peak}$ ), anaerobic capacity, and 60 min submaximal running tests. Fasted venous blood samples were drawn for the determination of triglyceride and glucose concentrations.

$VO_{2peak}$  increased in both FASTED ( $4.4 \pm 3.0\%$ ,  $p = 0.001$ ) and FED ( $4.6 \pm 4.2\%$ ,  $p = 0.017$ ), whereas maximal running velocity increased only in the FASTED ( $3.5 \pm 2.7\%$ ,  $p = 0.002$ ). Lactate concentrations in the anaerobic test after intervention were greater in FASTED than FED ( $p = 0.025$ – $0.041$ ). Running time in the anaerobic test was improved in FASTED (from  $64.1 \pm 15.6$ – $86.3 \pm 23.2$  s,  $p < 0.001$ ) but not in FED (from  $56.4 \pm 15.2$ – $66.9 \pm 21.3$  s,  $p = 0.099$ ). Substrate oxidation did not change after intervention in either of the groups ( $p = 0.052$ – $0.597$ ). Heart rate was lower in the submaximal running test in FASTED ( $p < 0.001$ ) but not in FED ( $p = 0.097$ ).

Training with periodised carbohydrate availability does not have any effect on substrate oxidation.

However, it seems to enhance the capacity to perform high-intensity exercise.

Keywords: Endurance; Nutrition; Metabolism; Aerobic fitness

- This training regime did not affect substrate oxidation, whether it was commenced with periodised or continuous carbohydrate availability.
- Training with periodised carbohydrate availability enhanced the capacity to perform a high-intensity exercise.

- It may also improve other endurance training adaptations such as lower heart rate during submaximal exercise.

## 1 Introduction

Recently, there has been an increased interest in endurance training with low carbohydrate (CHO) availability. It has been shown to promote greater changes in fat oxidation rates compared to training with normal or high CHO availability (Hansen et al., 2005; Hulston et al., 2010; Pilegaard et al., 2002; Yeo et al. 2008). However, these studies also showed decreased training intensity and have not demonstrated any superior improvement in performance compared to continuous CHO availability. Also, training with low CHO availability may increase susceptibility to illness and infection (Gleeson, Nieman, & Pedersen, 2004). That has led to the idea that high-intensity interval training (HIIT) is performed in the evening with high CHO availability followed by an overnight fast, and a low-intensity endurance exercise is performed with low CHO availability in the following morning. This approach is known as “train-high, sleep low” (Lane et al., 2015). This training-diet strategy enables high CHO availability for high-intensity exercise. In contrast, restricted CHO availability after HIIT and during low-intensity exercise together with depleted muscle glycogen stores may enhance metabolic signalling such as an increase in p53 phosphorylation and adenosine monophosphate-activated protein kinase activity associated with low CHO availability (Akerstrom et al., 2006; Bartlett et al., 2013; Civitarese, Hesselink, Russell, Ravussin, & Schrauwen, 2005; Cochran, Little, Tarnopolsky, & Gibala, 2010; Lane et al., 2015; Morton et al., 2009; Van Proeyen, Szlufcik, Nielens, Ramaekers, & Hespel, 2011; Wojtaszewski et al., 2003). Besides showing enhancement in cell signalling, a study by Bartlett et al. (2013) also showed that exercise intensity was not impaired when training was commenced with low CHO availability.

A study by Marquet et al. (2015) was the first to investigate this kind of training-diet strategy. They found that periodizing CHO intake enhanced performance and improved submaximal cycling efficiency without affecting fat oxidation. The performance was enhanced by the same magnitude in a study that investigated the short-term effect of periodised CHO intake with no changes in substrate

utilisation (Marquet et al., 2016). A study by Burke et al. (2017) compared the effect of a low CHO-high fat diet, periodised CHO availability, and high CHO availability on endurance training adaptations. Only periodised and chronic CHO groups improved their performance without having any changes in fat oxidation, whereas the low CHO-high fat group had an increase in fat oxidation leading to increased oxygen demand. Training with periodised CHO intake seems beneficial for improving performance; however, this enhancement does not seem to be due to improved fat metabolism (Marquet et al., 2015; Marquet et al., 2016). These previous studies have been done in highly trained athletes, and therefore, changes in fat oxidation might have been so minimal that they were not measurable (Hawley, 2014).

Thus, this study aimed to investigate whether periodizing CHO intake around specific training sessions would enhance endurance training adaptations in recreationally endurance-trained participants. The study consisted of two parts. First, an acute exercise experiment was conducted to examine the acute effect of periodised CHO intake on fat oxidation during low-intensity training (LIT). We hypothesised that the fat oxidation would be higher during LIT after an overnight fast than when consuming food, implying that there is lower CHO availability during LIT. Secondly, the main aim was to study whether a 4-week endurance training block with periodised CHO intake would lead to more beneficial endurance training adaptations than training with continuous CHO intake. We hypothesised that endurance training with periodised CHO availability would enhance endurance training adaptations to a greater extent than training with continuous CHO availability.

## 2 Methods

### 2.1 Participants

Participants were healthy males and females who had previous experience in a range of endurance-based sports such as orienteering, long-distance running, and football. Six participants took part in the acute exercise experiment (see below 2.2) (females: 5, males: 1, body mass:  $66.4 \pm 4.1$  kg, height:  $170.7 \pm 4.0$  cm, age:  $25 \pm 2$  yr, peak oxygen uptake ( $VO_{2peak}$ ):  $48.1 \pm 4.3$ ). Out of the six, five participants continued to take part in the 4-week training intervention. Before starting the training intervention, participants ( $n = 20$ ) were appointed to either the FASTED or the FED group based on their  $VO_{2peak}$ . During the intervention, three participants dropped out due to upper respiratory tract infections. Therefore 17 participants completed the study (FASTED =  $n: 9$ , females: 6, males: 3, body mass:  $67.5 \pm 2.8$  kg, height:  $171.5 \pm 2.1$ , age:  $28 \pm 2$  yr,  $VO_{2peak}$ :  $47.9 \pm 1.5$  ml/min/kg; FED =  $n: 8$ , females: 6, males: 2, body mass:  $71.7 \pm 2.5$  kg, height:  $171.0 \pm 3.3$ , age:  $27 \pm 2$  yr,  $VO_{2peak}$ :  $46.1 \pm 1.3$  ml/min/kg). Participants' health was ensured by delivering a health questionnaire before beginning the study. After explaining the purpose of the study and the possible risks involved, the participants signed a written consent form. The study was approved by the Research Ethics Committee of University of Jyväskylä.

### 2.2 Experimental Design

The study consisted of two parts: an acute exercise experiment and a chronic training intervention. The acute exercise experiment looked at the acute effect of the training-diet strategy on substrate metabolism. The chronic training intervention looked at the chronic effect of the training-diet strategy. There were two testing days. On day one  $VO_{2peak}$  test and an anaerobic test was performed with 3-5 h between them. On day two, 60 min running trial was completed.

### 2.3 Acute exercise experiment

Participants taking part in the acute exercise experiment performed first  $VO_{2peak}$  and anaerobic tests. Participants then completed in random order two HIIT-LIT combinations with different nutritional treatments in a cross-over design with 1–4 d intervals. Participants either performed HIIT in the evening and were restrained from eating until finishing LIT in the following morning (LOW) or ate after HIIT and before LIT (HIGH). Same running velocities were used during both the HIIT and LIT sessions.

*Substrate oxidation during aerobic exercise.* During the acute exercise experiment, LIT was performed in the laboratory where participants ran on a treadmill (OJK-KOMI, Telineyhtymä, Finland) for 60 min while their respiratory gasses were analysed (Oxygon Pro, Jaeger, Germany). The incline of the treadmill was at  $0.5^\circ$ . After the first 10 min, the treadmill was stopped, and blood lactate concentration was measured from the blood samples obtained from the participants' fingertips to ensure that intensity was correct (Lactate Scout+, EKF Diagnostic, Germany). Heart rate (HR, Polar V800, Finland) and the rate of perceived exertion (RPE) were recorded every 10 min. Running velocity was adjusted according to lactate and HR values during the first LIT. From respiratory gases, five-minute averages were further analysed at 15-20, 35-40, and 55-60 min of the exercise for measurements of oxygen uptake ( $VO_2$ ) and carbon dioxide production ( $VCO_2$ ) and calculation of respiratory exchange ratio (RER). Following equations were used to calculate whole-body CHO and fat oxidation (Péronnet & Massicotte, 1991):

$$CHO = 4.585 \times VCO_2 - 3.226 \times VO_2$$

$$Fat = 1.695 \times VO_2 - 1.701 \times VCO_2$$

## 2.4 Chronic training intervention

For the participants taking part only in the intervention, the two testing days were consecutive days or had one day between them. The participants taking part in the acute experiment completed testing day two after the acute experiment. The training period started 1–3 days after the second day of testing. The four-week training period included 16 training sessions where the combination of HIIT-LIT was performed twice a week with at least one day between them. HIIT was performed in the evening and LIT in the following morning. Post-testing was delivered 2–3 days after the last training session and included the same tests as pre-testing. During post-testing, the same running velocities were used in the anaerobic test and 60-min submaximal test, and the same starting velocity was used in  $VO_{2peak}$  as in pre-tests. All participants completed a three-day food diary two days before and on the first testing day, which was replicated during post-testing. Also, a four-day food diary was collected during the third week of training.

## 2.5 Endurance training protocols

The HIIT consisted of four sets of four minutes running at or above 95% of their  $VO_{2peak}$ , determined from the pre-testing and prescribed by running pace (min/km). This HIIT protocol was chosen since it has been shown to improve  $VO_{2max}$  (Helgerud et al., 2007) and is used as a training method by endurance athletes. During recovery the two-minute recovery period between sets, participants kept moving slowly or stood still. LIT consisted of running below the participants' aerobic threshold (HR determined from the  $VO_{2peak}$  test) for 60 min. After the 1–3 HIIT and LIT sessions, blood lactate concentrations were measured (Lactate Scout+, EKF Diagnostic, Germany) to ensure correct training intensities.

Supervised training sessions were performed outside on a flat surface at the specified times. There was at least one day between every HIIT-LIT combination. If required, participants were allowed to

do training sessions on their own. Participants were given appropriate guidelines and asked to report HR and running velocities from the training sessions.

Participants were allowed to continue with their usual training outside of the training days. Throughout the intervention, participants kept a record of their training (type and duration). Activities were divided into four categories; endurance training, strength training, fitness classes, and others included activities such as bouldering, yoga, aqua aerobics, and walking.

## 2.6 Nutritional manipulation

The FASTED group restrained from eating after HIIT until completing LIT the following morning (water consumption was allowed). However, they were instructed to consume the same number of calories and CHO during the day as usual. The FED group was instructed to consume food with 1-1.5 g of CHO per kilogram of body mass after cessation of the HIIT session and before LIT.

## 2.7 Testing

*VO<sub>2peak</sub> test.* Before the test, participants body mass and height were measured. A capillary blood sample was drawn from the fingertip to determine resting blood lactate concentration. The warm-up was delivered at a starting running velocity ranging from 6 to 9 km/h depending on the participants training background. The test consisted of three-minute workloads with a 1 km/h increase in speed after every workload until volitional exhaustion. The treadmill was stopped after every workload and fingertip capillary blood samples were drawn. HR and RPE were recorded on every workload. Moreover, blood samples were drawn one minute after the participant completed the test and after every three minutes during their 9-minute active recovery. Blood samples were analysed (Biosen S\_line Lab+ lactate analyzer, EKF Diagnostic, Germany) to determine blood lactate concentrations. Expired gases were analysed (Oxygon Pro, Jaeger, Germany) during the test, and VO<sub>2peak</sub> was

determined as the highest 30 s average of  $\text{VO}_2$ . Maximal HR ( $\text{HR}_{\text{max}}$ ) and maximal running velocity ( $v\text{VO}_{2\text{peak}}$ ) were obtained. Aerobic and anaerobic thresholds were determined by analysing changes in lactate concentrations and respiratory gases according to the Aunola and Rusko threshold concept (Aunola & Rusko, 1986). The same researcher determined the thresholds for all participants. Two submaximal workloads (SW1 = females: 9 km/h, males: 10 km/h; SW2 = females: 11 km/h, males: 12 km/h) were selected to investigate changes in HR, oxygen uptake ( $\text{VO}_2$ ),  $\text{VO}_2$  relative to  $\text{VO}_{2\text{peak}}$  ( $\% \text{VO}_{2\text{peak}}$ ), RER and lactate concentration.

*Anaerobic capacity test.* Participants warmed up for 5 minutes by running at 50% of their  $v\text{VO}_{2\text{peak}}$ . After this period, a mask was placed on them for the collection of expired gases. Participants then ran one minute at 60%, followed by two minutes at 80%, and then two minutes at 60% of their  $v\text{VO}_{2\text{peak}}$  before the speed was increased to 125% of their  $v\text{VO}_{2\text{peak}}$ . Participants ran for as long as possible. Three capillary blood samples were taken at 1, 3, and 5 minutes after finishing the test to determine lactate concentrations. Respiratory gases, HR, and total running time at 125% of their  $v\text{VO}_{2\text{peak}}$  was measured. Oxygen deficit was determined by calculating the difference between the actual values of oxygen uptake during the supramaximal workloads from this test and the estimated oxygen demand derived from the submaximal workloads from the  $\text{VO}_{2\text{peak}}$  test.

*60-minute running trial.* To determine fat and CHO metabolism during exercise, participants run on a treadmill for 60 min at 60% of their  $\text{VO}_{2\text{peak}}$  after an overnight fast. Respiratory gases were collected during the test. Five-minute averages were analysed at 15-20, 35-40, and 55-60 min of exercise. Measurements of  $\text{VO}_2$ ,  $\text{VCO}_2$ , and RER were recorded, and the equations from Péronnet and Massicotte (1991) were used to calculate fat and CHO oxidation rates.

*Venous blood samples.* Before the training intervention, antecubital venous blood samples were drawn from 13 volunteers after their overnight fast (FASTED: females=4, males=3, FED: females=5, males:1) of which glucose and triglyceride concentrations were determined. Blood samples were redrawn two to five days after the last training session. A photometric assay machine (Konelab 20, Vantaa, Finland) was used to analyse blood samples.

*Food diaries.* Participants were asked to complete a three-day food diary, where two days were recorded before the first testing day and one day during the first testing day. Participants then replicated the food diary before the post-testing as precisely as possible. A four-day food diary was also collected during the third week of the training period during the training days. Participants were given detailed instructions on how to fill the food diary correctly. All food diaries were analysed by using AivoDiet software (AivoFinland, Finland) by the same researcher. The measurement error of food diaries to estimate energy intake is approximately -15% (Posluna, Ruprich, & de Vries, 2009).

## 2.8 Statistical analysis

The Shapiro-Wilk test was used to test the normality of the data. The differences between substrate oxidation rates and HR in the acute experiment were determined by using a repeated measure analysis of variance (ANOVA). An independent t-test was used to find differences in pre-intervention measurements between groups. To find differences between pre- and post-testing values, repeated measure two-way ANOVA was used, whereas a one-way ANOVA was used to find differences in the changes between the two groups. Fisher's Least Significant Difference was used to specify the differences. An independent t-test was used to find differences in training between the two groups. If data was not normally distributed, Mann-Whitney U test and Wilcoxon signed-rank test were used to determine differences. A significant value of  $p < 0.05$  was chosen. Values are presented as mean  $\pm$  standard deviation.

## 3 Results

### 3.1 Pre-intervention values

No differences were found in any of the pre values between the two groups ( $p > 0.05$ ).

### 3.2 Body Mass

Body mass decreased significantly in FASTED (from  $67.5 \pm 8.4$  kg to  $66.4 \pm 8.2$  kg,  $p = 0.021$ ), whereas no change was observed in FED (from  $71.7 \pm 7.1$  kg to  $71.5 \pm 7.3$  kg,  $p = 0.799$ ). There were no differences between the groups ( $p = 0.114$ ).

### 3.3 Acute exercise experiment

The estimated total whole-body fat oxidation during 60-minute aerobic training was  $29.5 \pm 4.1$  g during LOW, which was significantly higher than during HIGH ( $18.3 \pm 4.7$  g,  $p < 0.001$ ). Correspondingly, the total average whole-body CHO oxidation was lower ( $76.8 \pm 30.0$  g) in LOW compared to HIGH ( $108.5 \pm 34.6$  g,  $p < 0.001$ ). No statistically significant differences ( $p = 0.166$ ) were observed in average HR between the LOW ( $146 \pm 6$  bpm) and HIGH ( $143 \pm 6$  bpm).

### 3.4 Chronic training intervention

#### 3.4.1 $VO_{2peak}$ test

Relative  $VO_{2peak}$  improved in FASTED  $4.4 \pm 3.0$  % from  $47.9 \pm 4.6$  to  $50.0 \pm 4.7$  ml/kg/min, ( $p = 0.001$ ) and in FED group  $4.6 \pm 4.2$  % from  $46.1 \pm 3.8$  to  $48.2 \pm 3.6$  ml/kg/min, ( $p = 0.017$ ) with no differences between the groups ( $p = 0.395$ ). Absolute  $VO_{2peak}$  also improved in both groups (FASTED: from  $3.2 \pm 0.5$  l/min to  $3.3 \pm 0.5$ ,  $p = 0.032$ , FED: from  $3.3 \pm 0.4$  to  $3.4 \pm 0.5$ ,  $p = 0.004$ ) with no differences between groups ( $p = 0.591$ ). FASTED improved in  $vVO_{2peak}$  ( $3.5 \pm 2.7$  %, from  $15.1 \pm 1.4$  km/h to  $15.6 \pm 1.3$  km/h,  $p = 0.002$ ) while no change was observed in FED ( $2.0 \pm 2.6$  %, from  $14.3 \pm 1.6$  km/h to  $14.5 \pm 1.7$  km/h,  $p = 0.056$ ). Running velocity at anaerobic threshold

improved in both FASTED ( $6.7 \pm 3.5$  % from  $12.1 \pm 1.5$  km/h to  $12.9 \pm 1.3$  km/h,  $p < 0.001$ ) and FED ( $6.1 \pm 5.3$  % from  $11.3 \pm 1.9$  km/h to  $12.0 \pm 1.9$ ,  $p = 0.013$ ) with no differences between the groups ( $p = 0.283$ ). Lactate concentrations after the  $\text{VO}_{2\text{peak}}$  test were higher after training in FASTED at each time point ( $p = 0.001$ - $0.023$ ), whereas no change was observed in the lactate concentrations in FED. There were no differences between the groups ( $p = 0.366$ - $0.889$ , Table 1).

Insert Table 1 here

HR at the two submaximal workloads (SW1: females 9 km/h and males 10 km/h, SW2: females 11 km/h and males 12 km/h) in the  $\text{VO}_{2\text{peak}}$  test decreased in both FASTED (SW1:  $p = 0.002$  and SW2:  $p = 0.004$ ) and FED (SW1:  $p = 0.020$  and SW2:  $p = 0.001$ ) with no differences between groups (SW1:  $p = 0.989$  and SW2:  $p = 0.641$ , Table 2). FASTED group had a decrease in  $\% \text{VO}_{2\text{peak}}$  at both submaximal workloads (SW1:  $p = 0.001$  and SW2:  $p = 0.006$ ), whereas in the FED group,  $\% \text{VO}_{2\text{peak}}$  decreased only at SW1 ( $p = 0.012$ ), and no change was found in the SW2 ( $p = 0.057$ , Table 2). No differences were observed between the groups (SW1:  $p = 0.212$  and SW2:  $p = 0.225$ ). No change was observed in lactate, RER, or  $\text{VO}_2$  (Table 2).

Insert Table 2 here

#### 3.4.2 Anaerobic test

Running time at 125% of the  $v\text{VO}_{2\text{peak}}$  improved in FASTED by  $34.2 \pm 13.0\%$  ( $24.3$ - $44.2$ ) (from  $64.1 \pm 15.6$  s to  $86.3 \pm 23.2$  s,  $p < 0.001$ ), whereas no statistically significant change was observed in the FED group ( $20.7 \pm 38.49\%$  ( $-11.5$ - $52.8$ ) from  $56.4 \pm 15.2$  to  $66.9 \pm 21.3$  s,  $p = 0.099$ ). No differences were observed between the groups ( $p = 0.093$ ). Statistically significant change was not observed in oxygen deficit in either of the groups (FASTED: from  $18.5 \pm 3.8$  ml/min/kg to  $18.9 \pm 5.5$  ml/min/kg,  $p = 0.441$  and FED: from  $22.8 \pm 5.6$  ml/min/kg to  $20.4 \pm 3.8$  ml/min/kg,  $p = 0.082$ ). After the intervention, lactate concentrations at each time point increased significantly only in the FASTED group ( $p = 0.001$ - $0.013$ ) and FASTED had higher values at each time point after the intervention than FED ( $p = 0.025$ - $0.041$ , Table 3).

Insert Table 3 here

### 3.4.3 60-minute running trial

No statistically significant changes were observed in RER (from  $0.84 \pm 0.04$  to  $0.86 \pm 0.04$ ,  $p = 0.154$  in FASTED and from  $0.85 \pm 0.04$  to  $0.86 \pm 0.03$ ,  $p = 0.075$  in FED) nor in substrate oxidations in either of the groups following the intervention (Fig. 1,  $p = 0.052-0.851$ ). The average HR decreased significantly in FASTED ( $6.5 \pm 1.5$  %, from  $139 \pm 6$  bpm to  $130 \pm 6$  bpm,  $p < 0.001$ ), whereas no changes were observed in FED ( $3.7 \pm 5.7$  %, from  $138 \pm 9$  bpm to  $133 \pm 7$  bpm,  $p = 0.097$ ), with no significant differences between the groups ( $p = 0.303$ ).

Insert Fig. 1 here

### 3.4.4 Blood parameters

Following the intervention, no statistically significant changes were observed in blood triglyceride concentrations (from  $0.91 \pm 0.44$  mmol/l to  $1.33 \pm 1.14$  mmol/l,  $p = 0.410$  in FASTED and from  $0.84 \pm 0.23$  mmol/l to  $0.79 \pm 0.28$  mmol/l,  $p = 0.600$  in FED) or in blood glucose concentration (from  $5.04 \pm 0.30$  mmol/l to  $4.97 \pm 0.51$  mmol/l,  $p = 0.682$  in FASTED and from  $4.82 \pm 0.43$  mmol/l to  $4.97 \pm 0.57$  mmol/l,  $p = 0.707$  in FED).

### 3.4.5 Nutrition

No statistically significant changes were observed in total energy intake between test and training collection in either group or between groups (FASTED: test  $1760 \pm 330$  kcal and training  $1910 \pm 330$  kcal,  $p = 0.201$ ; FED: test  $2120 \pm 610$  kcal and training  $2130 \pm 580$  kcal,  $p = 0.923$ ). No statistically significant differences were found in macronutrient intake between test and training collection in FASTED (protein  $1.2 \pm 0.4$  g/kg and  $1.4 \pm 0.4$  g/kg,  $p = 0.079$ ; CHO  $3.0 \pm 0.7$  g/kg and  $3.2 \pm 1.1$  g/kg,  $p = 0.226$  and fat  $0.9 \pm 0.2$  g/kg and  $1.0 \pm 0.2$  g/kg,  $p = 0.472$  training and test collection respectively) or in FED (protein  $1.3 \pm 0.4$  g/kg and  $1.3 \pm 0.4$  g/kg,  $p = 0.914$ ; CHO  $3.4 \pm 0.8$  g/kg and  $3.4 \pm 1.1$  g/kg,  $p = 0.927$  and fat  $1.0 \pm 0.4$  g/kg and  $1.1 \pm 0.3$  g/kg,  $p = 0.732$  training and test collection

respectively) nor between the groups ( $p = 0.413-0.741$ ). FED group consumed  $1.2 \pm 0.6$  g/kg of CHO after cessation of the HIIT session, and  $0.8 \pm 0.4$  g/kg before LIT during the training collection ( $n = 7$ ).

#### 3.4.6 Total training load

One participant from both groups completed 88% of the training included within the research, whereas everyone else completed all training sessions. There were no significant differences in total training amount (FASTED:  $634 \pm 306$  min and FED:  $673 \pm 202$  min,  $p = 0.888$ ) or different types of training outside of training involved with the research between the groups (endurance:  $369 \pm 263$  min and  $257 \pm 237$  min,  $p = 0.374$ ; strength:  $164 \pm 177$  min and  $113 \pm 120$  min,  $p = 0.504$ ; fitness classes:  $18 \pm 37$  min and  $152 \pm 255$  min,  $p = 0.277$ ; others:  $82 \pm 109$  min and  $150 \pm 169$  min,  $p = 0.423$  in FASTED and FED groups respectively).

## 4 DISCUSSION

The main findings in this study were that (1) substrate oxidation was not altered after the training intervention regardless of the nutritional approach and (2) the capacity to perform high-intensity exercise was higher after periodised CHO availability strategy.

### 4.1 Acute exercise experiment

Our first aim was to investigate whether remaining fasted after HIIT in the evening would cause greater fat oxidation during LIT in the following morning than when eating is not restricted. In the present study, fat oxidation was greater after LOW than HIGH. Hence, this suggests that the HIIT was intense enough to deplete at least some muscle glycogen stores and, therefore, the LIT was performed in low CHO availability. A previous study conducted by Bartlett et al. (2012) showed that the HIIT protocol of  $6 \times 3$  min at 90%  $VO_{2peak}$  with three-minute active recovery between the bouts depleted muscle glycogen  $\sim 30\%$ . Also, a study by Cochran et al. Cochran et al. (2010) showed that muscle glycogen stores were depleted by 30% after performing HIIT, including  $5 \times 4$  min at 90-95% of HR reserve, with a two-minute rest period. Based on these findings, it is likely that LIT was done with depleted glycogen content to create low CHO availability for the LIT session. These results were in accordance with our hypothesis.

### 4.2 Chronic training intervention

Our second aim was to examine whether periodising CHO availability around specific training sessions would enhance training adaptations in greater magnitude than when training with continuous CHO availability. Chronic training of HIIT in the evening followed by the LIT session the next morning, improved  $VO_{2peak}$  regardless of the nutritional approach. Other studies have shown similar findings. Van Proeyen et al. (2011) showed an increase in  $VO_{2peak}$  after six weeks of training in both

the overnight fasted and fed groups with no differences between them. Another study where participants trained either twice a day with or without glucose ingestion between the training sessions also showed an increase in  $VO_{2peak}$  regardless of the nutritional treatment (Morton et al., 2009). In our study, this training strategy also improved anaerobic threshold, time to exhaustion in the  $VO_{2peak}$  test, and decreased HR and  $VO_2$  relative to  $VO_{2peak}$  at submaximal workloads in both groups. Therefore, we can conclude that this training strategy, at least for four weeks, was enough to promote endurance-training adaptations regardless of the nutritional approach in previously recreational endurance-trained participants.

Restricting CHO availability before, during, and/or after training has been shown to improve fat oxidation (Hulston et al., 2010; Yeo et al. 2008) and enhance signalling pathways and enzymatic activity involved in the mechanisms to improve fat oxidation in trained and untrained participants (Akerstrom et al., 2006; Bartlett et al., 2013; Civitarese et al., 2005; Cochran et al., 2010; Lane et al., 2015; Morton et al., 2009; Van Proeyen et al., 2011; Wojtaszewski et al., 2003; Yeo et al. 2008). The training-diet strategy used in the present study may theoretically promote training benefits: it provides high CHO availability for high-intensity exercise and prolonged signalling responses after exercise and enhances metabolic signalling during prolonged low-intensity exercise. Therefore, this training-diet strategy should improve fat oxidation. However, no change in substrate oxidation was observed after the intervention regardless of the nutritional approach. This finding is supported by other studies examining the same training-diet strategy (Marquet et al., 2015; Marquet et al., 2016; ). Since there was no change in fat oxidation in either of the groups in the present study, we could speculate whether the training was intense enough to promote these changes. As proposed in the study by Lane et al. (2015) and Bartlett et al. (2013), enhancing some of these signalling pathways might require a certain magnitude of muscle glycogen depletion. Since muscle glycogen levels in loaded muscles were not measured, we cannot determine whether glycogen levels were depleted enough to promote

enhancement in the signalling pathways. Studies from Burke et al. (2017) and Marquet et al. (2015) did not show any effect on fat oxidation either. In these studies, the participants were highly trained athletes, and the experiment was three weeks long. Perhaps a longer intervention period may be required to observe statistically significant changes in fat oxidation with highly trained endurance athletes. Since we do not have data of female participants usage of hormonal contraception or menstrual cycle, the possible effect that females' hormonal status has on substrate utilisation during exercise should be taken into account when interpreting these results (Boisseau & Isacco, 2021; Elliott-Sale et al., 2021).

In the current study, body mass decreased only in the FASTED group after the intervention even though the energy balance was not significantly different between the treatments. (Iwayama et al., 2014) have shown that in an energy-balanced condition, 24-h fat oxidation increased when exercise performed before breakfast caused a significant transient energy deficit. Another study by Iwayama et al. (2015) also supports these findings. This could be a possible explanation why the body mass decreased only in the FASTED group in the current study.

Interestingly, the current training-diet strategy improved the capacity to perform high-intensity exercise. Only FASTED group improved running time in the anaerobic test and had significantly higher lactate concentrations after the intervention than FED. Furthermore, only the FASTED group improved  $vVO_{2peak}$  in the  $VO_{2peak}$  test and had higher lactate concentrations after the intervention. Both groups improved their  $VO_{2peak}$  after the intervention. However, only FASTED improved the  $vVO_{2peak}$  that could be explained, at least in part, by enhanced anaerobic capacity, indicated by improved running time in the anaerobic test. It has been shown that training with low CHO availability elevates resting muscle glycogen content (Hansen et al., 2005; Yeo et al. 2008). Hansen et al. (2005) suggested that training with low muscle glycogen availability stimulates glycogen

synthase signals, which regulates a glycogen synthesis leading to a greater muscle glycogen content. Thus, it could be suggested that a low CHO training-diet strategy enhanced glycolysis due to increased muscle glycogen content and/or enhancement in glycolysis. Since these factors were not measured in the present study, future studies are needed to investigate the underlying mechanisms to improve capacity to perform high-intensity exercise. When interpreting these results, we should take into account the possible placebo effect if participants in the FASTED group believed that they could perform better after the treatment. This can, at least in part, explain the improved ability to perform high-intensity exercise.

Previous studies have not found any changes in HR after training in either periodised or chronic CHO availability groups after the training period (Marquet et al., 2015) or have shown a trend of lower HR in both groups after training, but no differences between groups (Burke et al., 2017). In this study, HR was lower in both submaximal workloads at the  $VO_{2peak}$  test regardless of the nutritional approach. Oxygen uptake relative to  $VO_{2peak}$  was significantly lower in FASTED after intervention at the second submaximal workload and had a greater, although not statistically significant, decrease at the first submaximal workload. However, the average HR was lower during the 60-min running test only in the FASTED group after the training period. Therefore, these findings suggest that training with periodised CHO intake may promote beneficial adaptations in other endurance training parameters.

Our results support the practical train-low concept “the fuel for the work required” to promote training adaptations (Impey et al., 2016). According to this concept CHO availability should be manipulated day-to-day and meal-by-meal depending on the intensity and duration of the training session rather than implementing a chronic period of CHO restrictions.

### 4.3 Conclusion

The present study demonstrates that the training regime where HIIT was performed in the evening followed by low-intensity exercise the following morning did not affect substrate oxidation regardless of the nutritional approach. However, training with periodised CHO availability enhances the capacity to perform high-intensity exercise and seems to enhance other endurance training adaptations, such as lower heart rate in submaximal intensities in amateur and recreational athletes.

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**Declaration of interest****Conflict of interest**

The authors declare that they have no conflict of interest.

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Table 1. Blood lactate concentrations in the FESTED and FED groups before (PRE) and after (POST) training period during active recovery at +1, +4, +7, +10 -minute after finishing the  $VO_{2peak}$  test.

		+1 min	+4 min	+7 min	+10 min
FASTED	PRE (mmol/l)	10.6 ± 2.6	9.7 ± 3.0	7.7 ± 2.6	5.9 ± 3.4
	POST (mmol/l)	12.6 ± 2.2*	11.6 ± 2.6*	9.6 ± 2.7*	7.3 ± 2.6*
FED	PRE (mmol/l)	12.0 ± 2.8	10.5 ± 2.3	8.6 ± 2.4	7.3 ± 2.2
	POST (mmol/l)	11.7 ± 2.5	10.4 ± 2.9	8.3 ± 2.9	7.1 ± 2.6

Values are presented as mean ± SD. \*Significantly different from PRE (p<0.05)

Table 2. Oxygen consumption ( $\text{VO}_2$ ),  $\text{VO}_2$  relative to maximal oxygen uptake ( $\% \text{VO}_{2\text{peak}}$ ), respiratory exchange ratio (RER), heart rate (HR), and lactate concentration values at two submaximal workloads (SW1: female = 9 km/h, male = 10 km/h, SW2: female = 11 km/H, male = 12 km/h) before (PRE) and after (POST) intervention presented in the two experimental groups.

		PRE SW1	POST SW1	PRE SW2	POST SW2
$\text{VO}_2$ (ml/kg/min)	FASTED	33.9 ± 3.1	33.3 ± 3.2	39.8 ± 3.0	39.8 ± 2.8
	FED	34.0 ± 4.1	34.2 ± 4.1	40.5 ± 4.4	40.7 ± 4.7
$\% \text{VO}_{2\text{peak}}$	FASTED	71.0 ± 6.0	66.8 ± 6.4*	83.5 ± 6.6	80.1 ± 6.5*
	FED	73.8 ± 7.5	71.3 ± 7.5*	87.9 ± 7.3	84.7 ± 8.6
RER	FASTED	0.90 ± 0.04	0.90 ± 0.03	0.95 ± 0.03	0.95 ± 0.03
	FED	0.92 ± 0.05	0.93 ± 0.05	0.99 ± 0.07	0.98 ± 0.06
HR (bpm)	FASTED	158.4 ± 9.1	150.6 ± 5.7*	171.9 ± 9.9	165.8 ± 8.4*
	FED	157.4 ± 12.3	150.6 ± 14.3*	174.3 ± 11.4	168.1 ± 11.9*
Lactate concentration (mmol/l)	FASTED	1.6 ± 0.4	1.7 ± 0.4	2.6 ± 0.9	2.5 ± 0.7
	FED	2.2 ± 1.1	2.1 ± 1.2	4.0 ± 2.4	3.7 ± 2.6

Values are presented as mean ± SD. \*Significantly different from PRE ( $p < 0.05$ )

Table 3. Lactate concentrations before (PRE) and after (POST) training period and the change in lactate concentrations at 1, 3 and 5 minutes after the anaerobic capacity test for FASTED and FED groups.

		+1 min	+3 min	+5 min
FASTED	PRE (mmol/l)	8.6 ± 1.5	9.7 ± 2.2	10.0 ± 2.5
	POST (mmol/l)	10.8 ± 1.6* <sup>†</sup>	12.0 ± 1.9* <sup>†</sup>	12.7 ± 1.8* <sup>†</sup>
FED	PRE (mmol/l)	7.0 ± 2.2	8.3 ± 2.5	8.3 ± 2.6
	POST (mmol/l)	8.5 ± 2.2	9.5 ± 2.7	9.6 ± 3.0

Values are presented as mean ± SD. \*Significantly different from PRE (p<0.05) <sup>†</sup>Significantly different from FED (p<0.05)

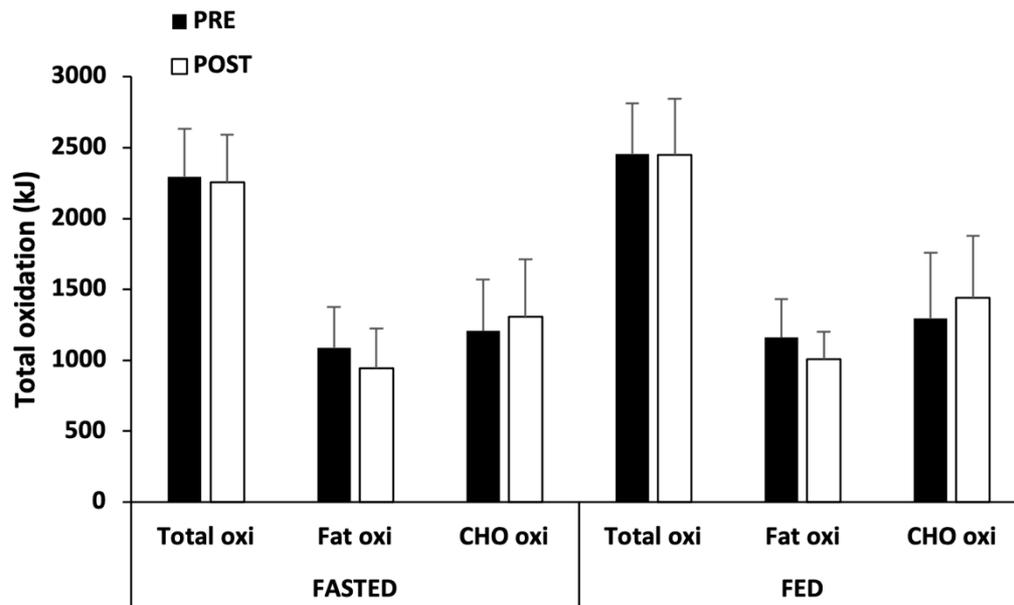


Figure 1. Differences in total oxidation (Total oxi), whole-body fat (fat oxi) and carbohydrate oxidation (CHO oxi) during 60-minute submaximal test before (PRE) and after (POST) four-week training intervention for both experimental groups. Values are presented as mean  $\pm$  SD