

**MANIPULATION OF CARBOHYDRATE AVAILABILITY TO PROMOTE
TRAINING ADAPTATIONS IN ENDURANCE TRAINED INDIVIDUALS**

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ABSTRACT

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Training with low CHO availability has been shown to improve fat oxidation which is important for endurance performance by sparing glycogen. Therefore, the aim was to investigate whether performing high intensity interval training (HIIT) in the evening, followed by overnight fast and commencing low intensity training (LIT) in the following morning with low CHO availability will enhance training adaptations. First, pilot study was delivered to explore the acute effect of this strategy on fat oxidation. Training intervention was then delivered to investigate the effect of this strategy on endurance training adaptations.

Subjects were endurance trained healthy males and females. Six subjects took part in the pilot study, which was delivered in crossover design. In that all subjects performed two HIIT-LIT combinations with staying either fasted after HIIT until finishing LIT or consumed food after HIIT and before LIT. Altogether 17 subjects completed training intervention, which included four-week training period, where HIIT-LIT training combination was performed twice a week. Subjects were divided into two groups: FASTED (stayed fasted after HIIT until finishing LIT) and FED (consumed food after HIIT and before LIT). Pre- and post-testing included VO_{2max} test, anaerobic test, 60 min submaximal test to evaluate substrate oxidation and venous blood samples were taken.

In the pilot study fat oxidation was higher during LIT ($p < 0.05$) after staying fasted than when consuming food after HIIT and before LIT. After the intervention VO_{2max} increased significantly in both groups (FASTED: $4 \pm 1\%$, FED: $5 \pm 2\%$, $p < 0.05$) with same magnitude, whereas maximal speed was greater only in the FASTED group ($p < 0.05$). Change in lactate concentrations were significantly higher in the FASTED than FED group after the VO_{2max} test ($p < 0.05$). The running time in the anaerobic test was improved in the FASTED group, but not in FED (from 64.1 ± 5.2 s to 86.3 ± 5.2 s and from 56.4 ± 5.4 to 66.9 ± 7.5 s, respectively). Substrate oxidation did not change after training period in either of the groups. HR was lower during submaximal test in the FASTED group after the intervention ($p < 0.05$), whereas no change was found in the FED group ($p > 0.05$).

In conclusion, training with periodised CHO availability around specific training sessions do not hinder training adaptations and enhances the capacity to perform high intensity exercise and may promote other adaptations. We could speculate that the enhanced capacity to perform high intensity exercise is due to elevated muscle glycogen stores and enhanced glycolytic enzymes, however future studies are needed to verify this.

Key words: endurance training, training low, carbohydrate availability, fat oxidation, HIIT

ABBREVIATIONS

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ATP	Adenosine triphosphate
CaMK	Calmodulin-dependent protein kinase
CHO	Carbohydrate
COX	Cytochrome <i>c</i> oxidase
COX4I1	Cytochrome <i>c</i> oxidase subunit 4 isoform 1
CS	Citrate synthase
FA	Fatty acid
HAD	3-hydroxyacyl-CoA dehydrogenase
HIIT	High intensity interval training
HR	Heart rate
HR _{max}	Maximal heart rate
IMTG	Intramuscular triglyceride
LIT	Low intensity training
MAP	Maximal aerobic power
PGC-1 α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
p38-MAPK	p38 mitogen-activated protein kinase
RER	Respiratory exchange ratio
RPE	Rate of Perceived Exertion
TCA	Tricarboxylic acid
VO ₂	Volume of oxygen
VO _{2max}	Maximal oxygen uptake
vVO _{2max}	Maximal speed
%VO _{2max}	Fractional utilization of VO _{2max}

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1 INTRODUCTION

Endurance is defined as an ability to maintain a given velocity or power output for longest possible time (Jones & Carter 2000). Therefore, endurance performance is affected on the ability to resynthesis adenosine triphosphate (ATP), which requires sufficient oxygen delivery to the working muscle and fuel supply from carbohydrates and fats (Jones & Carter 2000). Adaptations of endurance training enhances the delivery of oxygen from the atmospheric air to the mitochondria and improves metabolism.

During long endurance activities, the role of carbohydrate (CHO) availability is a key factor determining performance by being a key substrate for muscle metabolism and for central nervous system (Burke 2010). Unlike lipid stores, that can provide energy for hours or even for days (Burke & Hawley 2002), glycogen stores can be depleted when the exercise duration exceeds over 90 minutes (Costill et al. 1973). It has been shown that the performance in longer durations exercises is associated with the person's capacity to reserve their glycogen stores at sufficient levels right up to the finish line (Shulman & Rothman, 2001). This is also supported by the findings that fatigue during long duration exercise seems to be appearing together with depletion of muscle and liver glycogen stores (Coggan & Coyle, 1987) and that this fatigue can be delayed and therefore improve performance by following hyperglycaemic diet (Hawley et al. 1997) and/or consuming CHO during exercise (Karelis et al. 2010).

Therefore, the ability to oxidize fat is an important factor in endurance performance. Endurance training have been shown to improve fat oxidation at submaximal intensities and therefore sparing glycogen (Romijn et al. 1993). This enhancement in fat oxidation has been thought to be associated with enhanced mitochondrial volume (Holloszy 2008), enhanced activity of the lipolytic enzymes and reduction in the cellular signals activating the main glycolytic enzymes (Yeo et al. 2011).

During the last 20-30 years, consumption of high amounts of CHO before, during and after endurance training have been thought to be beneficial so that muscle glycogen stores are

fulfilled and that every training session is then performed under optimal conditions (Burke 2010). However, recently there have been evidences that training with low CHO availability will promote greater changes in fat oxidation rates than when training with normal or high CHO availability (Pilegaard et al. 2002; Hansen et al. 2005; Yeo et al. 2008; Hulston et al. 2010). This manipulation of CHO availability during some exercise sessions have been widely used to promote endurance training adaptations.

2 DETERMINANTS OF ENDURANCE PERFORMANCE

2.1 Maximal oxygen uptake

Maximal oxygen uptake (VO_{2max}) is the highest rate of oxygen during high intensity exercise that body can absorb and that can be used by muscles (Basset & Howley 2000). It has thought to be one of the determinants of endurance performance. The rate of oxygen supply to the muscle is limiting factor for VO_{2max} and therefore VO_{2max} is related to cardiac output which in turn depends on stroke volume (Spina 1999). Greater VO_{2max} during maximal exercise is results of greater cardiac output as well as greater extraction of oxygen by the exercising muscle (Jones & Carter 2000). Total blood hemoglobin content increases due to endurance training, which increases the oxygen carrying capacity of the blood.

The degree of which endurance training increases VO_{2max} depends on multiple factors. Main factors are the individuals starting level of fitness, intensity and duration of the training program, and frequency and duration of the single training session (Jones & Carter 2000). The optimal exercise volume and intensity to improve VO_{2max} is unknown, however the role of high intensity training has been shown to be important when wanting to improve VO_{2max} (Tabata et al. 1997). VO_{2max} has shown to improve rapidly on the onset of endurance training. This rapid increase in VO_{2max} as well rapid decrease in submaximal heart rate (HR) is partly due to increase in blood plasma increasing blood volume which leads to increased stroke volume during exercise (Convertino 1991). When training progresses, VO_{2max} will eventually level off and later improvements in performance are largely due to submaximal factors such as improvements in exercise economy and lactate threshold (Jones & Carter 2000).

Maximal cardiac output, capacity of pulmonary diffusion, bloods capacity to transport oxygen and /or capacity of muscles to consume oxygen are thought to be limiting factors of VO_{2max} . Cardiac output is defined as a product of HR and stroke volume. Bassett & Howley (2000) showed that improvement in VO_{2max} was more due to improved cardiac output than muscles improved capacity to extract oxygen. Even though increased cardiac output is important factor for improving VO_{2max} , not only improved local blood volume is adequate to enhance VO_{2max} .

Increase in mitochondrial capacity is needed to cause pressure gradient between muscle capillaries and intracellular gradient. Also increased capillary density within the active muscle is important for maintaining the red blood cells transit time through the muscle during high local flow rates. Pulmonary diffusion capacity can be limiting factor for highly trained athletes when maximal intensity exercise is performed. Trained athletes have higher cardiac output, which can lead to reduced transit time for red blood cells in the pulmonary capillaries, meaning that when red blood cells pass through lungs, not all might fix with oxygen. The content of hemoglobin in the blood also effects on VO_{2max} , since higher hemoglobin content will increase blood capacity to transport oxygen. (Hauswirth & Meuer 2012).

2.2 Lactate threshold

Lactate threshold is a point where blood lactate concentration exceeds resting level and this is a good predictor of endurance performance (Jones & Carter 2000). Lactate threshold can be improved through endurance training and this improvement enable individuals to sustain higher absolute or relative exercise intensity without accumulation of blood lactate (Jones & Carter 2000). Faster fatigue has been observed when exercising above lactate threshold, which is due to either quicker depletion of muscle glycogen (Jones & Carter 2000) or due to the effects of metabolic acidosis on contractile function (Sahlin 1992). Hence, changes in lactate threshold can be used a marker for improved endurance capacity.

2.3 Exercise economy

Exercise economy is defined as the energy demand for a given absolute exercise intensity (Saunders et al. 2004) and it can vary between individuals even though they have similar VO_{2max} . Greater exercise economy is thought to be beneficial for endurance performance, since less oxygen is needed for certain exercise intensity and therefore untrained individuals have shown not to have as good exercise economy as athletes (Morgan et al. 1995). Exercise economy seems to greatest at the velocities that they habitually train and therefore if the aim is to decrease oxygen cost for all training intensities, training should include variety of speeds (Jones & Carter 2000). Endurance training can improve exercise economy in multiple ways

by improving oxidative capacity of the muscles and changes in recruitment patterns of motor units (Coyle et al. 1992), decreasing ventilation and HR for the same exercise intensity (Madsen & Djurhuus 1998), and through technique improvements (Jones & Carter 2000). Increased fat utilisation can be partly responsible for these improvements since greater amount of oxygen required for fat metabolism than CHO metabolism.

3 ENDURANCE TRAINING ADAPTATIONS

Repeated bouts of exercise performed over period of time causes changes in physiology that leads to improved performance. The magnitude of the training response depends on the duration, intensity and frequency of exercise bout as well as training status and genetics of the individual (Jones & Carter 2000). Training at lactate threshold has shown to improve endurance capacity (Ghosh 2004) since accumulation of lactate does not compromise the high quality aerobic training stimulus (Jones & Carter 2000). Training at the higher intensities is effective way of stimulating increases in VO_{2max} (Londeree 1997). Interval training has commonly used for training at high intensities since it allows performing greater amount of work at higher intensities. Recently high intensity training (HIIT) has proven to be as effective as traditional continuous endurance training to promote endurance adaptations such as improved fat oxidation (Gibala et al. 2006; Bacon et al. 2013; Milanovic et al. 2015). Stepto et al. (1998) demonstrated that training exercise bouts of 3-6 minutes at ~85% of peak power output elicited the greatest improvement in endurance performance.

3.1 Cardiovascular adaptations

One of the most important endurance training adaptations is improvement in cardiac output, which is a product of stroke volume and HR. This means that heart will become more efficient and more effective to circulate blood around (Warburton & Bredin 2012). As stated earlier, changes in maximal cardiac output is mostly responsible for the improved VO_{2max} and exercise capacity. This change in maximal cardiac output is due to increase in maximal stroke volume. Endurance trained athletes have maximal cardiac outputs ranging from 32 to 40 L/min (Warburton & Bredin 2012), whereas same values for untrained individuals are around 20 to 25 L/min (Gledhill, Cox & Jamnik 1994; Krip, Jamnik & Warburton 1997). The differences in VO_{2max} between trained endurance athletes and untrained individuals is related to cardiac output.

Enhanced cardiac function is largely due to improvements in stroke volume and its determinants. Increase in stroke volume due to endurance training have been proven in

healthy individuals (Goodman, Liu & Green 2004). Stroke volume is maintained and even increased in endurance trained athletes during exercise from incremental to maximal intensity, indicating adaptations in oxygen transport (Warburton & Bredin 2012). Endurance training induced enhancement in stroke volume leading to enhancement in cardiac function seems to be due to combination of intra myocardial factors and extra myocardial factors (Levine 2008), including training induced hypervolemia, enhanced ventricular compliance and increased cardiac dimension (Warburton & Bredin 2012).

3.2 Metabolic adaptations

Every bout of endurance exercise causes changes in gene expression within the muscle and when these bouts are repeated frequently these changes in gene expression leads to chain of responses. These lead to changes in muscle characteristics which leads to endurance training adaptations such as increased capacity to oxidise fat, increase CHO and fat stores and shift substrate oxidation from CHO to fat. (Holliday & Jeukendrup 2012).

Oxidative capacity is enhanced due to increase in mitochondrial content and upregulation of its machinery. Activity and content of an enzyme 3-hydroxyacyl-CoA dehydrogenase (HAD) which has a role in β -oxidation cycle is increased due to endurance training (Burgomaster et al. 2008). Endurance training also enhance the upregulation of enzymes that are involved in the process of fatty acyl-CoA entering mitochondria that have thought to be rate limiting step for fat oxidation during exercise in high intensities (Holliday & Jeukendrup 2012). Tricarboxylic acid (TCA) cycle has also role of improving oxidative capacity and endurance training have been shown to enhance content and activity of enzymes involved in TCA cycle (Holliday & Jeukendrup 2012).

As well as increased capacity to oxidase fat, endurance training increases CHO and fat storages. Endurance training has shown to increase muscle and liver glycogen stores. This seems to be due to enhanced activity of glycogen synthase within a muscle (Chris-Roberts et al. 2004) and increase in glucose transporter protein (GLUT-4) content which enhances the capacity of glucose uptake (Greiwe et al. 1999; Hansen et al. 2005). Intramuscular

triglyceride (IMTG) stores have shown to be increased due to endurance training together with increased fat oxidation (Tarnopolsky et al. 2007). However, it is not clear whether the elevated IMTG stores caused greater IMTG oxidation.

Endurance training causes shift in substrate oxidation from CHO to fat. Increased oxidation capacity causes less disturbance in homeostasis and therefore there is less metabolic stress signalling. These signals of metabolic stress regulate the rate of glycolysis. AMP activates glycogen phosphorylase and therefore low AMP concentrations will inhibit increase in rate of glycogenolysis. AMP also regulates AMPK which stimulates GLUT-4 translocation reducing the transport of glucose into muscle cell. These mechanism leads to downregulation of glycolytic utilisation of CHO and increase the role of fat as a fuel during submaximal exercise. (Holliday & Jeukendrup 2012).

Although we know that fat oxidation is increased due to endurance training, it is still unclear what is the source of this additional oxidised fat. As said earlier IMTG content in the muscle is increased due to endurance training, however its role of being this additional fuel source is unclear (Tarnopolsky et al. 2007). Its oxidation has shown to increase due to training (Phillips et al. 1996) but also has shown not to change due to training (Kiens et al. 1993). Fatty acid (FA) uptake and utilisation capacity has also shown to enhance due to endurance training. It was shown that fat utilisation was increased and this was accompanied by increased uptake of FA in untrained leg versus trained leg whereas no change in IMTG oxidation was not found (Kiens et al. 1993). Also, endurance training has shown to enhance mRNA for fatty acid translocase (FAT/CD36) and FAT/CD36 protein content which was also accompanied with increased uptake of FA (Tunstall et al. 2002).

3.3 Molecular adaptations

Endurance training stimulates multiple adaptations in skeletal muscle, where increases in mitochondrial mass is the most important one. Since mitochondria play a crucial role in fat oxidation, increased mitochondrial mass enables individuals to exercise longer duration at higher intensities (Bartlett et al. 2015). The increased mitochondrial content that accompanies

exercise training ensures that exercise in the trained state induces less perturbations to metabolic homeostasis, that is smaller decreases in ATP, phosphocreatine and muscle glycogen utilisation and smaller increases in adenosine diphosphate (ADP), adenosine monophosphate (AMP), inorganic phosphate and muscle lactate (Bartlett et al. 2015). Mitochondria are increased in size and number through process called mitochondrial biogenesis. It is a complex process and the precise molecular mechanism are not yet fully understood.

There is a close relationship between mitochondrial content and endurance capacity and it has been showed that highly trained individuals have 3-4 times higher mitochondrial content than untrained individuals (Costill et al. 1976; Hoppler 1990). Well trained individuals do not only have high VO_{2max} , they also have high fractional utilisation of VO_{2max} ($\%VO_{2max}$). The $\%VO_{2max}$ is closely related to muscle aerobic capacity and mitochondrial content, since higher mitochondrial content can increase fat utilisation and reduce formation of lactic acid at a given submaximal intensity (Basset & Howley 2000). Therefore, they are able to maintain high volume of oxygen (VO_2) for longer periods.

Exercise has shown to enhance mitochondrial biogenesis and the enhanced activity of Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) is thought to be one of the regulators (Hollooszy 2008). PGC-1 α gene expression, protein activity and the migration to mitochondria and nucleus is upregulated by exercise (Little et al. 2011; Baar 2014). Transcription and activity of PGC-1 α is regulated due to exercise through protein kinases AMP-activated protein kinase (AMPK), p38 mitogen-activated protein kinase (p38-MAPK) and calmodulin-dependent protein kinase (CaMK) (Kang & Li Ji 2012). In turn these protein kinases are regulated during exercise through contractile-induced stressors such as AMPK/ATP ratio and decreased energy availability (Bartlett et al. 2015).

In an animal study done by Saleem and colleagues (2009) tumour suppression protein p53 was knocked out showed that p53 may also be a potential regulator of mitochondrial content and exercise performance. Studies looking at acute exercise response on p53 confirm that it

has a role in regulating exercise-induced mitochondrial biogenesis (Bartlett et al. 2012; Philp et al. 2011; Saleem & Hood 2013).

4 TRAINING WITH LOW CHO AVAILABILITY

Training with low CHO availability has been shown to enhance fat oxidation in greater extent than when training with normal CHO availability (Yeo et al. 2008; Hulston et al. 2010; Hawley & Morton 2014). The underlining molecular mechanisms that augments these adaptations when training with low CHO availability are still not fully understood by this day. However, the activity of AMPK (Yeo et al. 2010) and P38-MAPK (Cochran et al. 2010) has shown to be affected by the availability of CHO and it is known that AMPK and p38-MAPK regulate activity of PGC-1 α (Kang & Li Ji 2012), which is thought to be the master regulator of mitochondrial biogenesis (Holloszy 2008). There are multiple ways of reducing CHO availability for training which are represented in the Table 1. Reduced CHO availability can be achieved by reducing endogenous or exogenous sources of CHO. Reducing endogenous sources means depleting the muscle glycogen stores and exogenous sources can be reduced by training after overnight fast and/or restricting ingestion of CHO during and/or after training.

4.1 Endogenous CHO availability and training adaptations

Hansen et al. (2005) were first to look at how training with low CHO availability effect on training adaptations. In this study, previously untrained male subjects took part in 10-week knee extensor training program where one leg was trained once daily and other leg was trained twice a day every second day with two-hour recovery between exercises with restricted CHO consumption. Both legs performed the same amount of total work but training twice a day protocol lead the leg perform the second session with 50% less muscle glycogen availability. They found that the time till exhaustion, resting muscle glycogen concentration and citrate synthase (CS) activity were enhanced when training twice a day compared with training once a day.

TABLE 1. Practical strategies to reduce carbohydrate availability to promote molecular adaptations to endurance-based training session.

Exercise diet strategy	Outcome		References
Chronically low CHO diet (CHO intake less than requirements for training are)	Endogenous and exogenous CHO availability reduced for all training sessions	May impair absolute training intensities May reduce immune function or emphasise immunosuppression that occurs post exercise	Gleeson et al. (2004)
Twice per day training (withholding CHO intake between training sessions performed in the same day)	Endogenous and exogenous CHO availability reduced for the muscle during second session	May improve training intensities May reduce immune function or emphasise immunosuppression that occurs post exercise	Hansen et al. (2005), Hulston et al. (2010), Morton et al. (2009), Yeo et al. (2008)
Training after overnight fast	Exogenous CHO availability reduced for muscle for specific training session Endogenous CHO availability may be reduced if recovery from previous days' training have not been optimal	May reduce immune function or emphasise immunosuppression that occurs post exercise	Van Proeyen et al. (2011)
Withholding CHO within first few hours of recovery	Adequate CHO availability for the muscle for the specific session enabling train intensively but augment the cell-signaling response in the targeted post-exercise recovery phase.	Could interrupt refueling for following training session if CHO intake is reduced rather than delayed May reduce immune function or emphasise immunosuppression that occurs post exercise	Pilegaard et al. (2005)
'Sleep low/train low'	Endogenous CHO stores depleted by evening session which is followed by overnight fast and training session on the next morning. Represent combination of all the above strategies to provide a prolonged period of CHO restriction before (i.e. overnight) during and after the morning training	May impair absolute training intensities May reduce immune function or emphasise immunosuppression that occurs post exercise	Bartlett et al. (2013)

Adapted from Bartlett, Hawley & Morton (2015)

Study done in highly trained cyclist by Yeo et al. (2008) used the similar training model where one group performed training twice a day (LOW) and other group trained once per day (HIGH) over three weeks. The training consisted of steady state cycling at 70% of VO_{2peak} for 100 minute and high intensity interval training of 8x5min at maximal effort with 1-minute

recovery between each exercise bout (HIIT). HIGH group alternated these training regimes daily, whereas LOW group performed steady state training in the morning and HIIT 1-2 hours after. They found that training twice a day resulted greater increases in the resting muscle glycogen content, activity of CS and β -hydroxyacyl-CoA dehydrogenase (β -HAD), content of cytochrome *c* oxidase (COX) subunit IV and whole-body fat oxidation rates during submaximal exercise than when training once a day. These changes occurred even though the training intensity during HIIT was decreased around 8% in the first two weeks for LOW group. However, these metabolic and enzymatic changes did not translate into enhanced endurance performance when compared with HIGH group. Hulston et al. (2010) replicated this same study design with addition of isotope tracers. They showed that training with reduced glycogen stores increases intramuscular triglyceride oxidation during exercise which is most likely due to increases in protein content of both β -HAD and lipid transporter, FAT/CD36. Nevertheless, no greater improvement in performance was not observed in the LOW group in this occasion either.

4.2 Exogenous CHO availability and training adaptations

Training with low exogenous CHO availability has also shown to be a potent stimulus to enhance these training adaptations. Feeding of CHO before, during and after exercise have shown to decrease abundance of AMPK (Civitarese et al. 2005; Akerstrom et al. 2006). Morton et al. (2009) also demonstrated that the enhancements in oxidative adaptations when training twice a day were dampened when exogenous glucose was ingested before and during the second training session. Bartlett and the colleagues (2013) demonstrated that ingestion of CHO rich meal before the exercise and provision of CHO during exercise and recovery decreased the signalling of p53. They also showed that restricting CHO intake during exercise and recovery the phosphorylation of p53 was three times higher than when consuming CHO during exercise and recovery. Regular training in fasted state has also shown to decrease exercise-induced glycogen breakdown, increase lipid transport proteins and enhance activity of CS and β -HAD (Van Proeyen et al. 2011). Nonetheless of these changes, no differences were found between fasted and fed state in VO_{2max} and cycling time-trial performance after the training period (Van Proeyen et al. 2011).

These studies show that training with low endogenous or exogenous CHO availability promotes oxidative training adaptations and improve the capacity to oxidise fat during exercise. However, training with reduced CHO availability seems to reduce the intensity of training and these adaptations does not seem to translate into improved endurance performance.

4.3 Periodised CHO availability

As we can see, training intensity have shown to be reduced during the low CHO availability high intensity training session and that it is know that high CHO availability is desirable when training in high intensities. This has led to an idea where HIIT is performed in the evening with high CHO availability followed overnight fast and performing a low intensity exercise in the following morning with low CHO availability. This training-diet strategy enables high CHO availability for high intensity exercise, whereas restricted CHO availability after HIIT and during low intensity exercise together with depleted muscle glycogen stores will enhance metabolic signalling associated with low CHO availability.

Previous studies investigating the timing of CHO availability around specific training sessions has showed that periodised CHO availability enhances endurance performance. Marquet et al. (2015) was first to investigate this strategy of altering the timing of CHO availability in trained triathletes over three-week period. Here they commenced six exercises over four consecutive days: three HIIT sessions and three low intensity sessions. HIIT consisted either running (6x5 min at individual 10 km intensity with 1 min rest between sets) or cycling (8x5min at 85% of maximal aerobic power (MAP) with 1 min rest between sets) and low intensity training was cycling (60 min at 65% of MAP). This study was first to show improvements in endurance performance in endurance-trained athletes when integrating training with low CHO availability. Training with altering CHO availability enhanced 10 km running performance, submaximal cycling efficiency was improved and Rate of Perceived Exertion (RPE) was lower during continuous exercise. They also showed that body mass and body fat mass was lower after training with altering CHO intake. (Marquet et al. 2015).

Another study from Marquet et al. (2016) investigated the short-term effect of periodised CHO intake on performance. Trained cyclist commenced six training session over one week involving three HIIT sessions (8x5 min at 85% of MAP with 1 min active recovery) and three low-moderate intensity sessions (60 min at 65% of MAP). After one-week, no changes in substrate utilisation were found in either of the groups and performance in 20 km time trial improved significantly in periodised CHO intake group compared to control group. The improvement in performance was same magnitude as found in study by Marquet at al. (2015) and the improvement was related to differences in pacing strategies. From here we can see that even as little as six training sessions with periodised CHO availability seemed to improve performance compared to chronic CHO availability, implicating that this type of training strategy can be beneficial in long term as well. (Marquet et al. 2016).

Burke et al. (2016) compared the effect of low CHO high fat diet, periodised CHO intake (alternating low and high CHO availability) and high CHO availability (CHO consumed before, during and after) has on endurance performance and metabolism. The study consisted of three-week intensified training period on elite race walkers. They found that fat oxidation was not enhanced after training in either of periodised nor chronic CHO availability groups, whereas high fat diet had significant increase in fat oxidation. This increased fat oxidation then lead to increased oxygen demand for a given walking speed. There was no improvement in performance in high fat diet, whereas both periodised and chronic CHO availability groups had significant improvement in performance with no differences between the groups. Hence, this study found no significant benefit form periodizing CHO availability, however it did show to be more beneficial than training with high fat diet. (Burke et al. 2016).

5 RESEARCH QUESTIONS AND HYPOTHESIS

As we can see training in high intensities with low CHO availability may reduce training intensity (Yeo et al. 2008; Hulston et al. 2010) and may increase susceptibility to illness and infection (Gleeson, Nieman and Pedersen 2004). Also, these strategies have not shown to enhance endurance performance which may be partly explained by the reduced training intensities. This has led to the idea of performing high intensity interval training (HIIT) in the evening with normal CHO availability, remaining fasted overnight and performing a prolonged submaximal training session in the following morning. Performing HIIT with normal CHO availability will ensure the high intensity training, whereas the restricted CHO availability after HIIT and performing the submaximal training with low CHO availability will enhance metabolic signaling. Previous studies investigating this type of training-diet strategy have not explored the effect of periodised CHO availability on anaerobic capacity.

Therefore, we are interested to investigate whether this training-diet protocol where CHO availability is periodised around specific training sessions will enhance endurance training adaptations. First, we are going to investigate whether staying fasted after HIIT will promote greater fat oxidation during LIT than when consuming food after HIIT and before LIT in the following morning. HIIT training have shown to deplete muscle glycogen stores (Bartlett et al. 2012; Cochran et al. 2010). Also, Bartlett et al. (2013) showed that when training with reduced muscle glycogen content which was achieved by performing HIIT (6x3min at 90% VO_{2max}), the amount of fat that is oxidised is greater than when training with high CHO availability. Therefore, we hypothesis that when exercising after an overnight fast, there will be greater oxidation of fat during submaximal exercise than when training with normal CHO availability.

Secondly, we are going to investigate whether chronic training with periodised CHO availability will enhance training adaptations greater amount than when training with chronic CHO availability. Previous studies have demonstrated that chronic training with low CHO will improve fat oxidation (Hansen et al. 2005; Yeo et al. 2008; Hulston et al. 2010). Also, increases in several mitochondrial signalling molecules and mitochondrial enzymes has also

been exhibited when training have been performed with reduced CHO availability (Bartlett et al. 2013) and when restricting CHO intake during the first hours of recovery (Pilegaard et al. 2005) or using the combination of these two strategies (Marquet et al. 2016). Therefore, we hypothesis that chronic training with periodised CHO availability will promote greater training adaptations than when training with chronic CHO availability.

6 METHODS

6.1 Subjects

Subjects were both healthy males and females who had various training backgrounds in endurance training. Endurance sports included orienteering, football, running, triathlon, gaelic football and cross-country skiing. All together 21 subjects participated in the study that were recruited through advertising in University of Jyväskylä, email advertising in different sport clubs in Jyväskylä area and in social media. Six subjects participated in the pilot study (Table 2) and out of the six, five subjects continued to participate in the intervention. All together 20 subjects participated in the intervention where three dropped out due to upper respiratory tract infection (Table 3). Before testing subjects were asked to fill pre-screening questionnaire to ensure their health. After explaining the purpose of the study and possible risks involved the subjects signed a written consent form. Before training period, subjects were divided into two groups, FED (high CHO availability by consumed food after HIIT and before aerobic training) and FASTED (periodised CHO availability by remaining from eating after HIIT until finishing aerobic training), based on their VO_{2max} values. The study was approved by the Research Ethics Committee of University of Jyväskylä.

TABLE 2. Subject characteristics before pilot study.

n	Females	Males	Age (yr)	Body mass (kg)	Height (cm)
6	5	1	25±1.9	66.4±4.1	170.7±4.0

Data is presented as mean±SE.

TABLE 3. Characteristics before pre-testing of the subjects taking part on pre-testing (A) and finishing the research (B). Data is represented in two groups: FASTED and FED. The FASTED group remained from eating after high intensity interval training (HIIT) until finishing low intensity training in the following morning whereas the FED group consumed food after HIIT and before low intensity training.

	Group	n	Females	Males	Age (yr)	Body mass (kg)	Height (cm)	VO _{2max} (ml/min/kg)
A	FASTED	10	7	3	28±2	67.9±2.5	171.5±1.9	47.6±1.4
	FED	10	7	3	28±2	73.1±2.6	173.9±3.5	46.4±1.2
B	FASTED	9	6	3	28±2	67.5±2.8	171.5±2.1	47.9±1.5
	FED	8	6	2	27±2	71.7±2.5	171.0±3.3	46.1±1.3

Data is presented as mean±SE.

6.2 Overview

The study consisted of two parts: pilot study and intervention. During the pilot study, acute effect of the training on substrate metabolism was studied and the intervention part looked at the chronic effect of the training intervention (Figure 1). There were two testing days where during the first one VO_{2max} test was delivered and then 3-5 hours later anaerobic test was done (Figure 2). The 60-minute running trial at ~60% of VO_{2max} was done on testing day two (Figure 2).

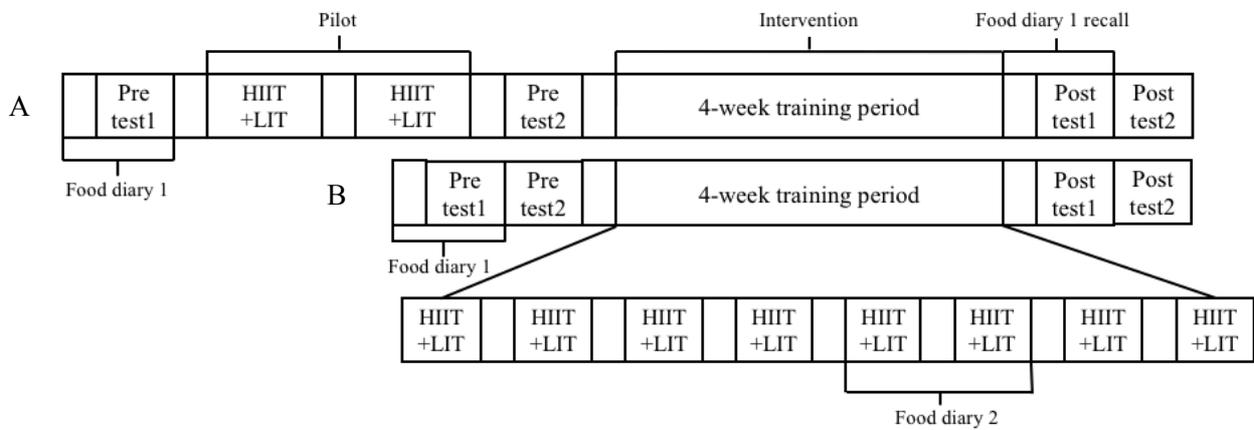


FIGURE 1. Overview of the study, where shows the design for subjects participating on both parts of the study (A) and the design for the subjects participating on the part two (B). First part and Pre test1 and Post test1 included VO_{2max} test and anaerobic test and Pre test2 and Post test2 included 60min submaximal test. HIIT+LIT represent training where subjects did high intensity interval training (HIIT) in the evening at or above 95% of their VO_{2max} , which was followed by low intensity training (LIT) below their aerobic threshold in the following morning.

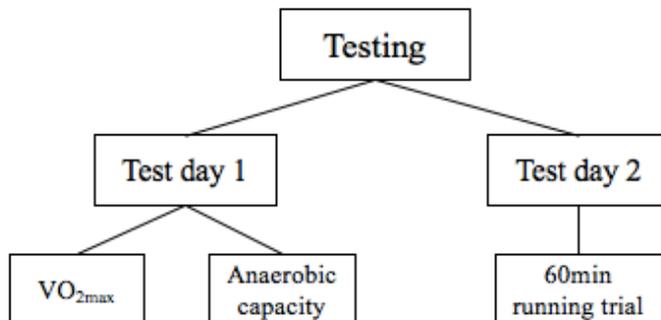


FIGURE 2. Overview of the testing days.

6.2.1 Pilot

Pilot study was done in cross-over design, where all subjects did two HIIT session followed by an aerobic training with two different nutritional treatments. First VO_{2max} test and

anaerobic test was delivered. From the VO_{2max} test, aerobic threshold and 95% of VO_{2max} was determined for defining correct training intensities. After that subjects took part in two treatments where HIIT was done in the evening which was followed by aerobic training in the following morning. In randomised order, subjects either remained from eating after HIIT until finishing the aerobic training in the morning (LOW) or ate after HIIT and before aerobic training (HIGH) and then repeated the training with different nutritional approach. After both treatments subjects performed 60-minute submaximal test and then started training intervention.

Substrate oxidation during aerobic exercise. During the pilot, aerobic training was delivered in the laboratory where subjects run on a treadmill below their aerobic threshold, while respiratory gasses were analysed. Incline of the treadmill was at 0.5°. After 10 minutes, the treadmill was stopped, and lactate concentration was measured from the fingertip to ensure that intensity was correct. HR and RPE was recorded after every 10 minutes. Velocity was adjusted according to lactate and HR values. After the 60 minutes, blood sample from a fingertip was taken to measure the lactate concentration. Subjects were allowed to listen music during the test. From respiratory gases, five-minute averages were further analysed at 15-20, 35-40 and 55-60 minutes of the exercise for measurements of VO_2 and VCO_2 and calculation of respiratory exchange ratio (RER). Following equations were used to calculate whole body fat and CHO oxidation (Peronnet and Massicotte, 1991):

$$\text{CHO: } 4.585 \times VCO_2 - 3.226 \times VO_2$$

$$\text{Fat: } 1.695 \times VO_2 - 1.701 \times VCO_2$$

6.2.2 Intervention

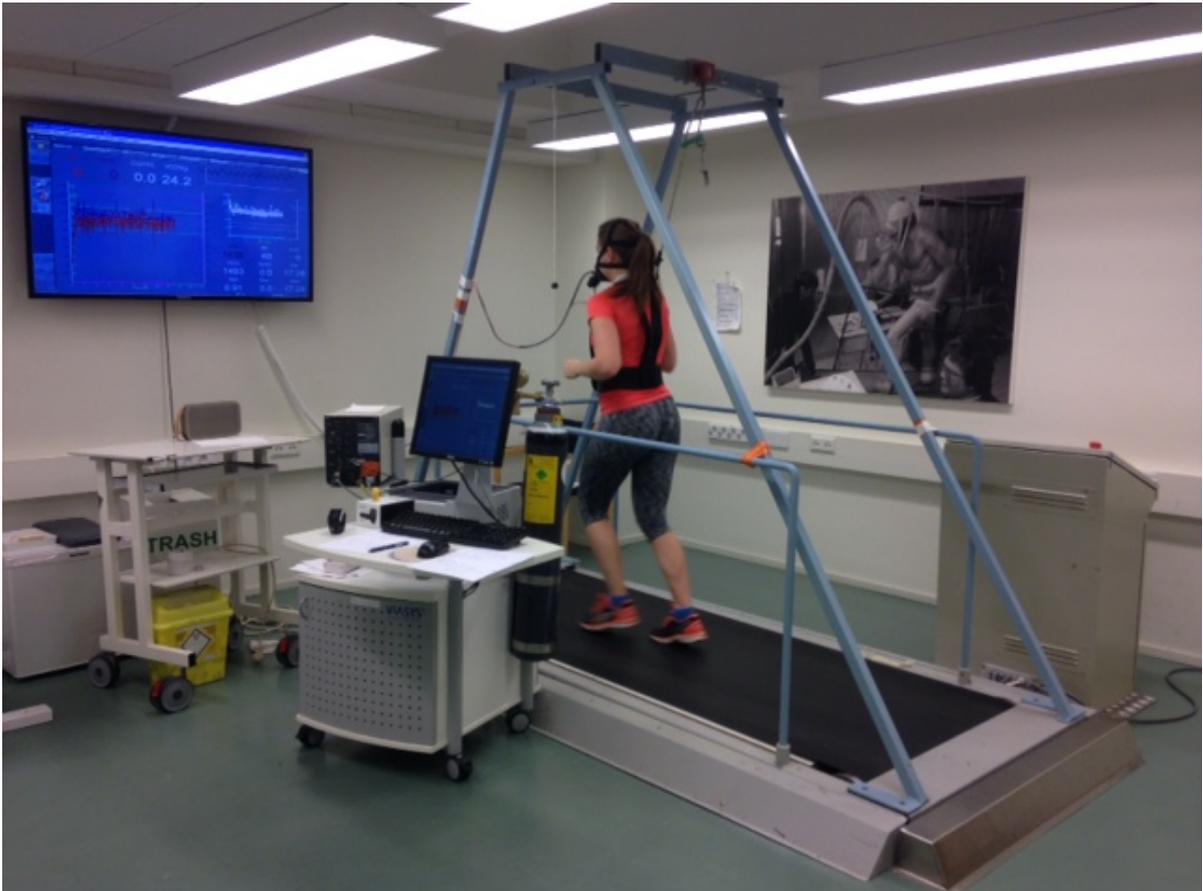
The participants taking part only on the intervention had same two testing days as the ones in part one. The testing days were either consecutive days or had one day between them. Participants taking part on both parts started the training period right after first part. Before the training period, subjects were divided into two different groups based on their VO_{2max} ,

where they either remained from eating after HIIT until finishing aerobic training (FASTED) or ate after HIIT and before the aerobic training (FED). Training period started 1-3 days after testing day two. Training period was four weeks long, which included two HIIT sessions in the evening and two aerobic training sessions in the following morning. Therefore, overall 16 training sessions was done over the four-weeks.

Post-testing was done 1-3 days after last training day (>48h after last training session). Post-testing included the same tests as pre-testing, where the anaerobic and 60-minute submaximal tests were done at the same velocities as in pre-test and same starting velocity was used in the VO_{2max} test both pre- and post-test.

6.3 Testing overview

All testing was delivered in the University of Jyväskylä laboratory. Same treadmill (OJK-KOMI, Telineyhtymä, Finland), gas analyser (Oxygon Pro, Jaeger, Germany) and HR monitor (Polar V800, Finland) were used (Picture 1). Breath-by-breath method was used in every expired gas measurement and the gas analyser was calibrated before every measurement. Lactate concentration was measured by using capillary blood samples during all testing and analysed by using lactate analyser (Biosen S_line Lab+ lactate analyzer, EKF Diagnostic, Germany). During training and during aerobic training in the first part lactate analyser (Lactate Scout+, EKF Diagnostic, Germany) was used to determine lactate concentrations. Before the first test subjects' height and body mass (Philips, HF 351/00, China) was measured.



PICTURE 1. Laboratory set up.

6.3.1 $\text{VO}_{2\text{max}}$ test

Before starting the test, blood sample was taken from the fingertip for determination of resting lactate concentration and five-minute warm-up at the starting speed was delivered. Starting velocity was chosen based on the participants training background and interviews. Starting velocity ranged from 6 to 9 km/h. After the warm-up mask was placed for measurements of respiratory gases. Participants were wearing a safety harness at all times when running on the treadmill.

The test consisted of three-minute workloads, with 1km/h increases in velocity after every workload. Incline of the treadmill was at 0.5° . After each workload treadmill was stopped and blood sample was taken from the fingertip for determination of blood lactate concentration. Time of taking blood samples were included into the next workload and if it took more than 40 seconds, one minute was added on the next workload. The velocity was increased by 1

km/h after every workload. Average HR was recorded during the first 15 seconds on the last 30 seconds of each workload. RPE was recorded after every workload. Participants were advised to run until volitional exhaustion and the test was determined after participants signalled so. Verbal encouragement was given to participants during the last minutes of the test. After the test was finished, blood samples were taken from the fingertip after one minute. Participants then started an active recovery at the starting velocity for nine minutes of where blood samples were taken after every three minutes.

From the expired gases, 30 second average was analysed and VO_{2max} was determined as the highest VO_2 value during the test. Maximal HR (HR_{max}) and maximal speed (vVO_{2max}) was derived from this test. Aerobic and anaerobic thresholds were determined from these results by looking at changes in lactate concentrations and respiratory gases.

6.3.2 Anaerobic capacity test

Anaerobic capacity was determined by measuring oxygen deficit. The subjects warmed-up for 5 minutes at 50% of their vVO_{2max} after which the mask was placed on subject for collection of expired gases. Subjects then run one minute at 60% then at 80% for two minutes and then two minutes at 60% of their vVO_{2max} before increasing the speed to 125% of their vVO_{2max} . Incline of the treadmill was at 0.5° . Subjects were advised to run as long as they possible. Verbal encouragements were given to participants during the test. Total time at 125% vVO_{2max} , respiratory gasses and HR were recorded.

6.3.3 60-minute running trial at ~ 60% VO_{2max}

To measure fat and CHO metabolism during exercise, 60-minute submaximal running test was delivered. Subjects run on a treadmill for 60 minutes at their ~60% of their VO_{2max} . Incline of the treadmill was at 0.5° . HR and RPE was recorded every 10 minutes during the test. Subjects were allowed to listen music during the test. Respiratory gases were collected during the test where from five-minute averages were further analysed at 15-20, 35-40 and 55-60 minutes of exercise for measurements of VO_2 and VCO_2 and calculation of RER. The

same equations from Peronnet and Massicotte (1991) was used to calculate fat and CHO oxidations than used in the pilot study.

6.3.4 Venous blood samples

Before or during the first week of the training period, venous blood samples were taken from 13 subjects (FASTED=7, FED=6) to determine glucose and FFA concentrations in the blood. Blood samples were then retaken after the training period. Since two of the subjects had to drop out from the study, only 11 samples were further analysed. Photometric assay machine (Konelab 20, Vantaa, Finland) was used to analyse blood samples. Equation by Dill and Costill (1974) was used to calculate the change in plasma volume.

6.4 Food diaries

Subjects were asked to complete three-day food diary, where two days were recorded before the first testing day and one day during the first testing day. Subjects then replicated the food diary before the post-testing as precisely as possible. Four-day food diary was also collected during the third week of the training period during the training days. Participants were advised to write down the timing, content and the amount of all food and drinks consumed during the day as precisely as possible. All food diaries were analysed by same researcher by using Aivodiet software.

6.5 Training

Training consisted of HIIT session in the evening which was followed by aerobic training on the following morning. All training was done running. Before the HIIT subjects conducted their own warm-up consisting of running and dynamic stretches. The HIIT consisted of 4x4min sets with two-minute recovery between each set at or above 95% of their VO_{2max} . During recovery subjects slowly walked back to starting point, kept slightly moving around or were standing still. After the HIIT, subjects delivered their own cool-down. Aerobic training was done on the following morning where participants run below their aerobic threshold for

60 minutes. After the first 1-3 sessions lactate concentrations were measured to ensure correct intensities of the training.

Subjects were recommended to take part on supervised training session, however opportunity to do some or all training sessions on their own was also allowed if needed/required. Subjects were given a choice of two training groups that were training on different days. All supervised trainings were done outside on a flat surface and HIIT sessions started from 6.45-7.30 pm and aerobic sessions started at 7.00 am. In the case of the training was done on their own, subjects were given appropriate guidelines and were asked to report HR and running velocities form the training sessions. Training diary was recorded during the training period and subjects were advised not to do any endurance training on the training days included in the study.

6.6 Nutritional manipulation

Subjects were divided into two groups based on their VO_{2max} before the training period. The FASTED group remained from eating after HIIT until finishing aerobic training in the following morning. The FED group consumed food after HIIT as well as before the aerobic training in the following morning. The FASTED group was not allowed to consume anything else expect water during the fasting, however they were advised to consume the same amount of CHO during the day as usual. The FED group were advised to eat at least 1-1.5g of CHO per kilogram of body mass after training and before the morning training. Some subjects were struggling to eat in the morning huge amounts of CHO, therefore they were advised to eat as much as possible.

6.7 Data analysis

Data was analysed by using IBM SPSS software. Shapiro-Wilkinson test was used to test the normality of the data. The differences between substrate oxidation rates in the pilot was determined by using a paired t-test. To find differences between pre- and post-testing values paired t-test was used whereas t-test was used to find differences in the changes between the

two groups. Mann-Whitney U test and Wilcoxon signed-rank test was used to determine differences if data was not normally distributed. Significant value of $p < 0.05$ was chosen.

7 RESULTS

7.1 Pilot study

During the aerobic training, RER was significantly lower when commencing the training LOW than HIGH ($p < 0.05$, Table 4). Estimated total whole-body fat oxidation during 60-minute aerobic training was 29.5 ± 1.7 g during LOW which was significantly higher than during HIGH which was 18.3 ± 1.9 g ($p < 0.05$, Figure 3). Total average whole-body CHO oxidation was 76.8 ± 12.2 g and 108.5 ± 14.1 g in LOW and HIGH respectively, where LOW had significantly lower value than HIGH ($p < 0.05$, Figure 3).

TABLE 4. Values of respiratory exchange ratio (RER) during 60-minute aerobic training represented in two treatments: LOW (remained from eating after high intensity interval training (HIIT) in the evening until finishing an aerobic exercise in the following morning) and HIGH (consumed food after HIIT and before aerobic training).

	15-20 min	35-40 min	55-60 min
LOW	0.85 ± 0.01	0.84 ± 0.01	0.83 ± 0.01
HIGH	$0.92 \pm 0.01^*$	$0.90 \pm 0.01^*$	$0.88 \pm 0.01^*$

Values are presented as mean \pm SE. *Significantly different than LOW ($p < 0.05$).

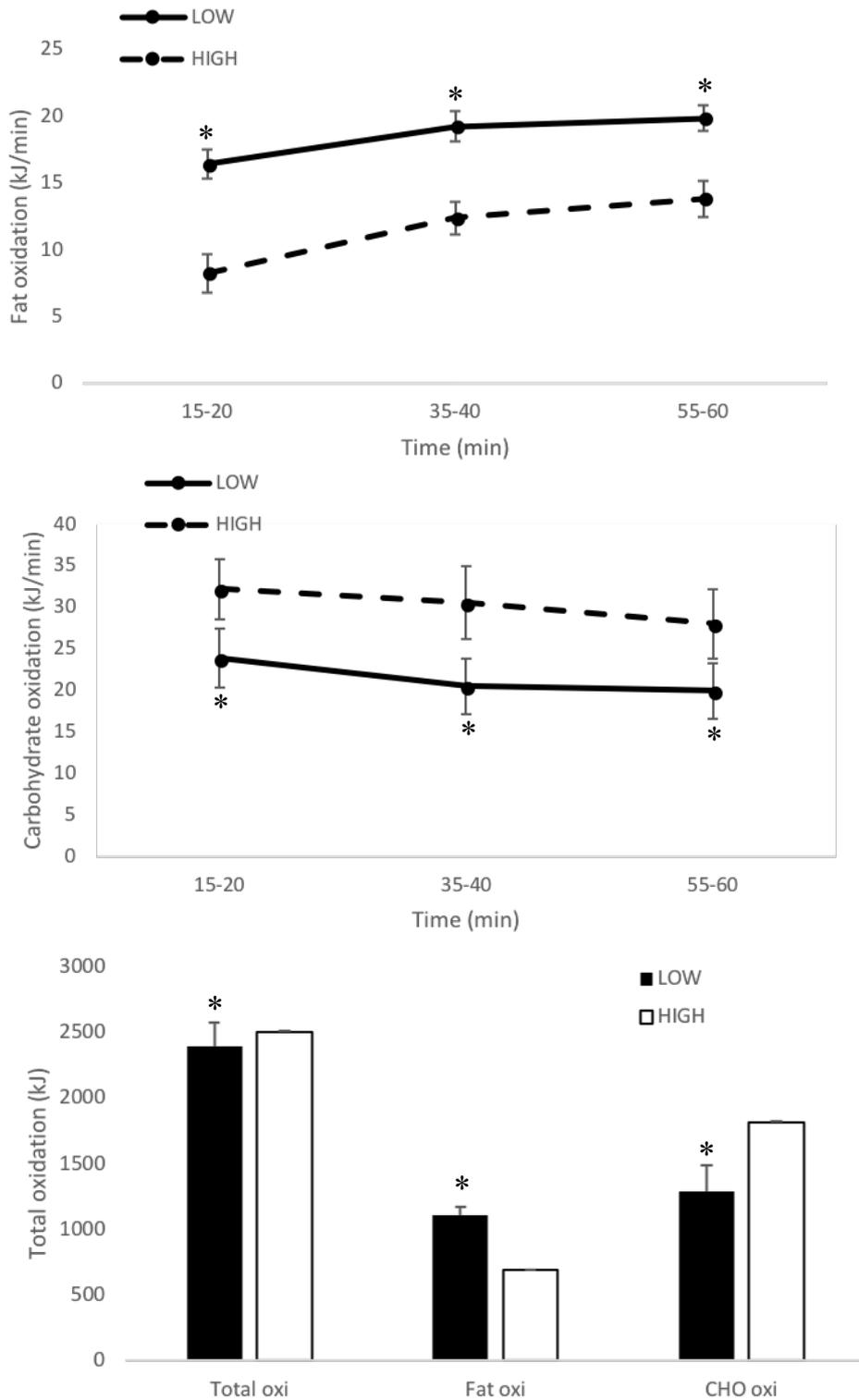


FIGURE 3. Differences in whole body fat oxidation (fat oxi), carbohydrate oxidation (CHO oxi) and total oxidation (Total oxi) during aerobic exercise represented in two treatments: LOW (subjects remained from eating after high intensity interval training session until finishing a 60-minute aerobic training session on the following morning) and HIGH (subjects consumed food after HIIT and before aerobic training session). Values are presented in mean±SE. *Significantly different from HIGH ($p < 0.05$).

Average HR was 146 ± 2 bpm during LOW and 143 ± 2 bpm during HIGH with no differences between the two treatments ($p > 0.05$, Figure 4). There were no differences in the average RPE between LOW and HIGH (11 ± 0.6 and 10 ± 0.6 respectively, $p > 0.05$).

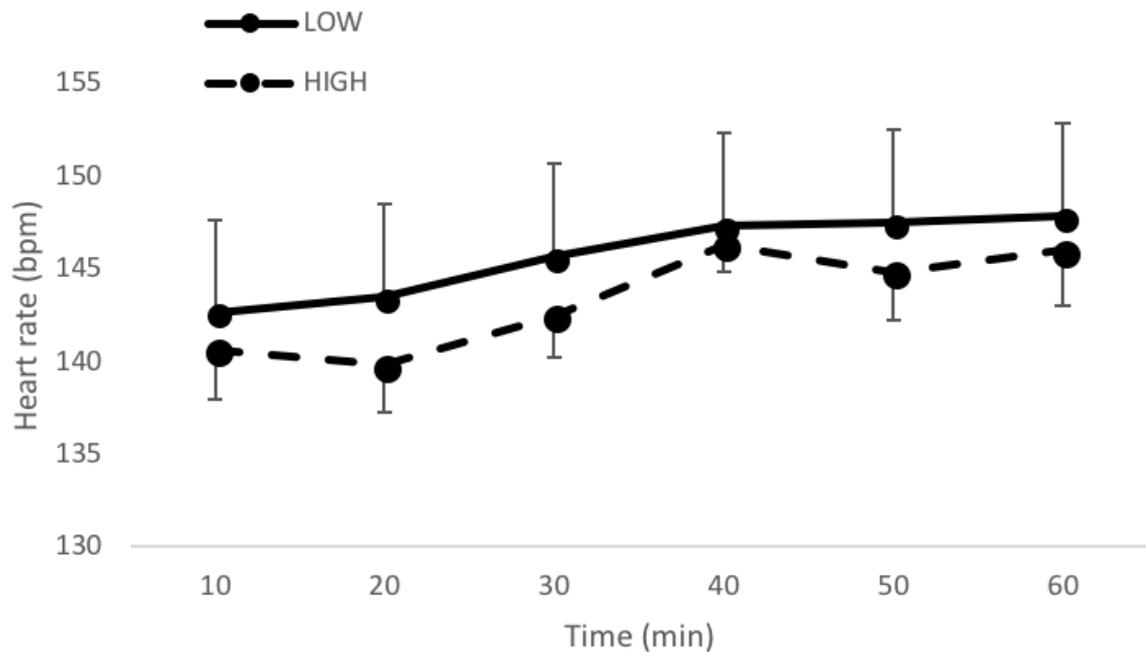


FIGURE 4. Heart rate at 10, 20, 30, 40, 50 and 60 -minute time point during 60-minute aerobic training session. Values are represented by two treatments: LOW (subjects remained from eating after high intensity interval training session until finishing a 60-minute aerobic training session on the following morning) and HIGH (subjects consumed food after HIIT and before aerobic training session). Values are presented in mean \pm SE.

7.2 Intervention

7.2.1 VO_{2max} test

Before the four-week training period, there were no differences in VO_{2max} between the FASTED and FED groups prior training ($p > 0.05$). Both groups had significant improvement on their VO_{2max} after the four-week intervention, where FASTED improved from 47.9 ± 1.5 to 50.0 ± 1.6 ml/kg/min and the FED group improved from 46.1 ± 1.3 to 48.2 ± 1.3 ml/kg/min

(Figure 5A). The FASTED group had increase of $4\pm 1\%$ in VO_{2max} , whereas the FED group had increase of $5\pm 2\%$.

There were no differences in vVO_{2max} between FASTED and FED before the training period ($p>0.05$). The FASTED group had a significant improvement in vVO_{2max} from 15.1 ± 0.5 km/h to 15.6 ± 0.4 km/h, whereas the FED group did not have any significant changes from 14.5 ± 0.6 km/h to 14.3 ± 0.6 km/h (Figure 5B). However, there were no differences between the groups ($p>0.05$).

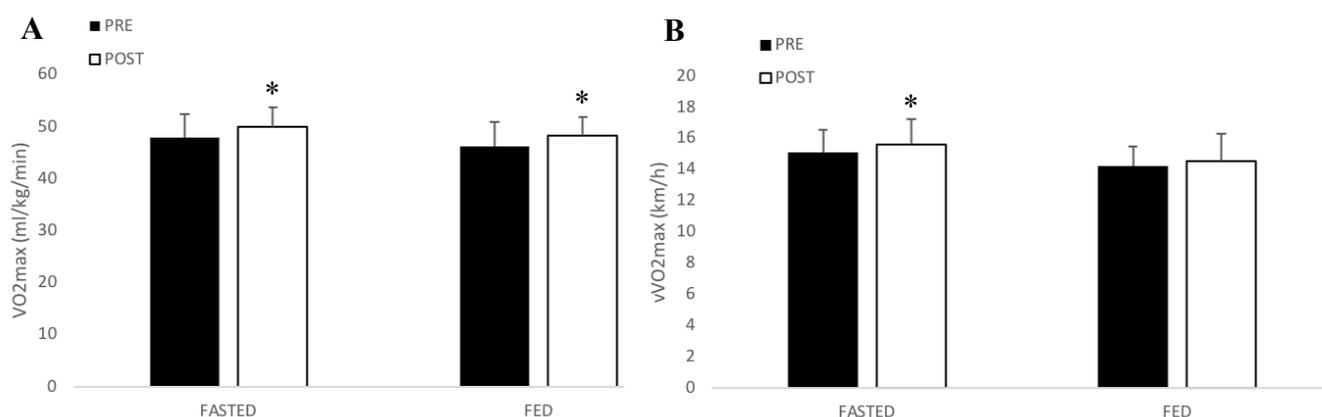


FIGURE 5. Maximal oxygen uptake (VO_{2max}) (A) and maximum speed (vVO_{2max}) (B) values from the VO_{2max} test before (PRE) and after (POST) four-week training period. Data is presented in two experimental groups, where one group remained from eating after high intensity interval training (HIIT) until finishing an aerobic training in the following morning (FASTED) and other group consumed food after HIIT and before aerobic training (FED). Values are presented as mean \pm SD. *Significantly different from PRE ($p<0.05$)

Lactate concentrations during active recovery were significantly higher after training period in the FASTED group at every time point ($p<0.05$), whereas no difference was found in the FED group values ($p>0.05$). The change in lactate concentration was significantly higher in the FASTED group compared to FED at all time points ($p<0.05$, Table 5).

TABLE 5. Change in lactate concentrations during active recovery at +1, +4, +7, +10 -minute time points after finishing the VO_{2max} test after four-week training intervention. Values are

presented in two groups, where one group remained from eating after high intensity interval training (HIIT) in the evening and before aerobic training in the following morning (FASTED) and other group consumed food after HIIT and before aerobic training (FED).

	+1	+4	+7	+10
FASTED	2.0±0.4*	2.0±0.5*	1.9±0.5*	1.3±0.5*
FED	-0.3±0.4	-0.1±0.5	-0.3±0.4	-0.2±0.3

Values are presented as mean±SE. *Significantly different from FED (p<0.05)

7.2.2 Anaerobic test

There were no differences at the time at 125% of the vVO_{2max} between FASTED and FED at pre-test (p>0.05). Running time at 125% of the vVO_{2max} was significantly greater after the training period in the FASTED group (from 64.1±5.2 s to 86.3±5.2 s), whereas there was no significant change in the time in the FED group (from 56.4±5.4 to 66.9±7.5 s). However, there were no differences between the groups after the training period (p>0.05, Figure 6A).

Before the training intervention, there were no differences between the groups in oxygen deficit (p>0.05). After the four-week training intervention, neither of the groups did not have significant changes in the values of oxygen deficit (FASTED from 18.5±1.3 ml/min/kg to 18.9±1.8 ml/min/kg and FED from 22.8±2.0 ml/min/kg to 20.4±1.3 ml/min/kg, Figure 6B).

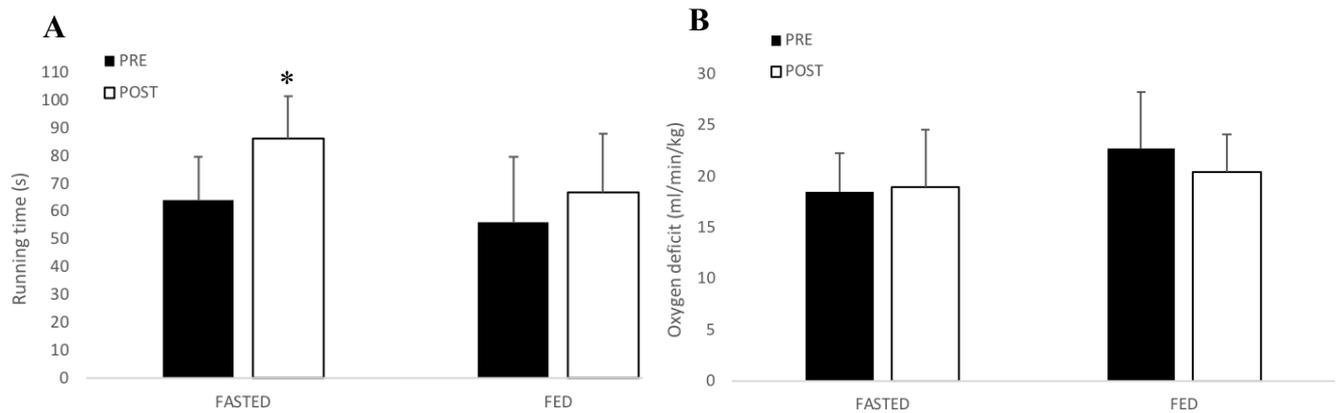


FIGURE 6. Running time at 125% of maximum speed from the VO_{2max} test (A) and oxygen deficit (B) values from the anaerobic test before (PRE) and after (POST) training intervention. Values are represented in two experimental groups: FASTED (remain from eating after HIIT until finishing aerobic training in the following morning) and FED (consume food after HIIT and before aerobic training). Values are presented as mean \pm SD. *Significantly different from PRE ($p < 0.05$).

Lactate concentrations at every time point were significantly different after training period in the FASTED group ($p < 0.05$), whereas no differences were not observed in the FED group at any time point ($p > 0.05$). There were no differences in the change of lactate concentrations between the groups ($p > 0.05$, Table 6).

TABLE 6. Change in lactate concentrations at 1, 3 and 5 minutes after four-week training period, where the FASTED group stayed fasted after high intensity interval training (HIIT) until finishing aerobic training in the following morning and where FED consumed food after HIIT and before aerobic training.

	+1 min	+3 min	+5 min
FASTED	2.1 \pm 0.7	2.2 \pm 0.4	2.7 \pm 0.7
FED	1.5 \pm 1.0	1.2 \pm 0.9	1.3 \pm 1.0

Values are presented as mean \pm SE

7.2.3 60-minute submaximal test

There was no change in VO_2 after the training period in either of the groups at any time point, except in the FASTED group at the second time point, where VO_2 values were lower after training period ($p < 0.05$, Table 7). No differences between the groups were found (Table 7). Average RER was not different between the groups before training period ($p > 0.05$) and it did not change significantly after the training period (FASTED: from 0.84 ± 0.01 to 0.86 ± 0.01 and FED: from 0.85 ± 0.01 to 0.86 ± 0.01). Substrate oxidation did not change after training intervention in either of groups and no significant differences were not found between the groups (Figure 7).

TABLE 7. Values of oxygen consumption during 60-minute submaximal test before (PRE) and after (POST) four-week training intervention. Values are represented in two groups, where one group stayed fasted after high intensity interval training (HIIT) until finishing a aerobic training in the following morning (FASTED) and one group consumed food after HIIT and before aerobic exercise (FED).

		15-20 min	35-40 min	55-60 min
FASTED	PRE	1.85 ± 0.09	1.87 ± 0.09	1.87 ± 0.09
	POST	1.80 ± 0.09	$1.82 \pm 0.09^*$	1.82 ± 0.08
FED	PRE	2.00 ± 0.10	2.00 ± 0.09	2.00 ± 0.10
	POST	1.94 ± 0.11	2.00 ± 0.10	2.00 ± 0.10

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

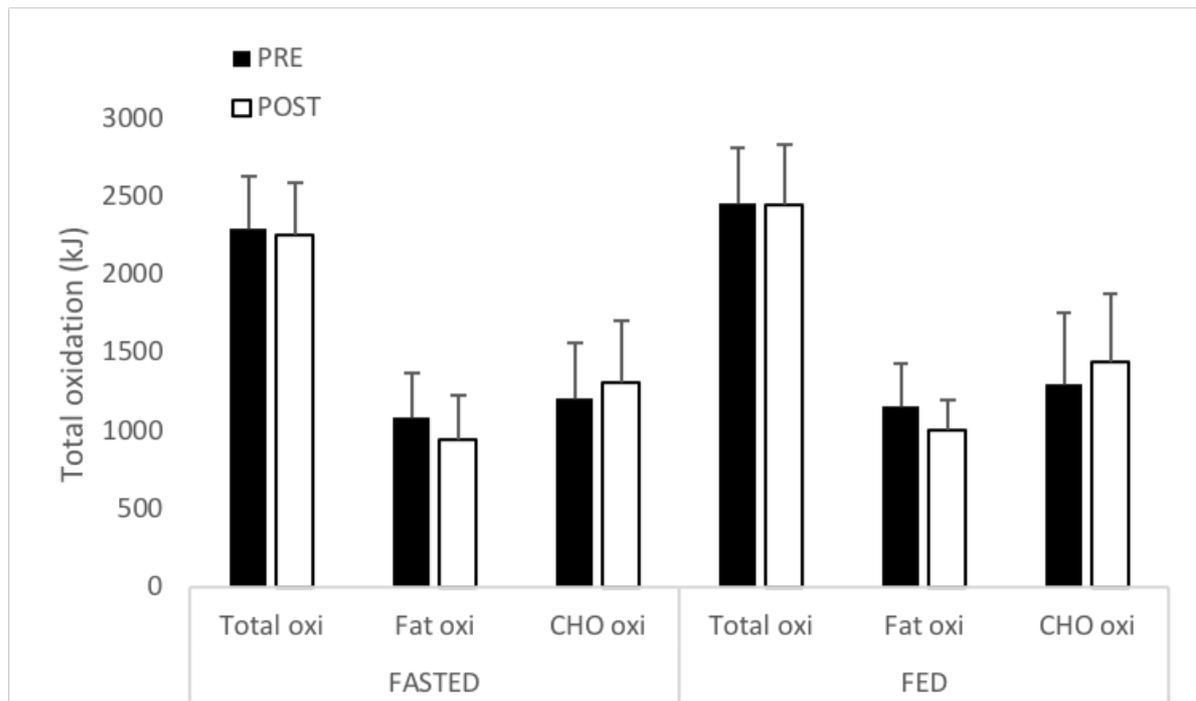


FIGURE 7. Differences in total oxidation (Total oxi), whole body fat (fat oxi) and carbohydrate oxidation (CHO oxi) during 60-minute submaximal test before (PRE) and after (POST) four-week training intervention. Data is represented in two groups: FASTED (remained from eating after high intensity interval training (HIIT) until finishing an aerobic exercise in the following morning) and FED (consumed food after HIIT and before aerobic training). Values are presented as mean±SD.

Average HR was not significantly different between the FASTED and FED during the 60-minute submaximal test before the training period ($p>0.05$). After the training period, FASTED average HR decreased significantly from 139 ± 2 bpm to 130 ± 2 bpm ($p<0.05$), whereas the FED group did not have any significant change in average HR (from 138 ± 3 bpm to 133 ± 2 bpm, $p>0.05$, Figure 8). However, there were no significant differences between the groups during post measurements ($p>0.05$).

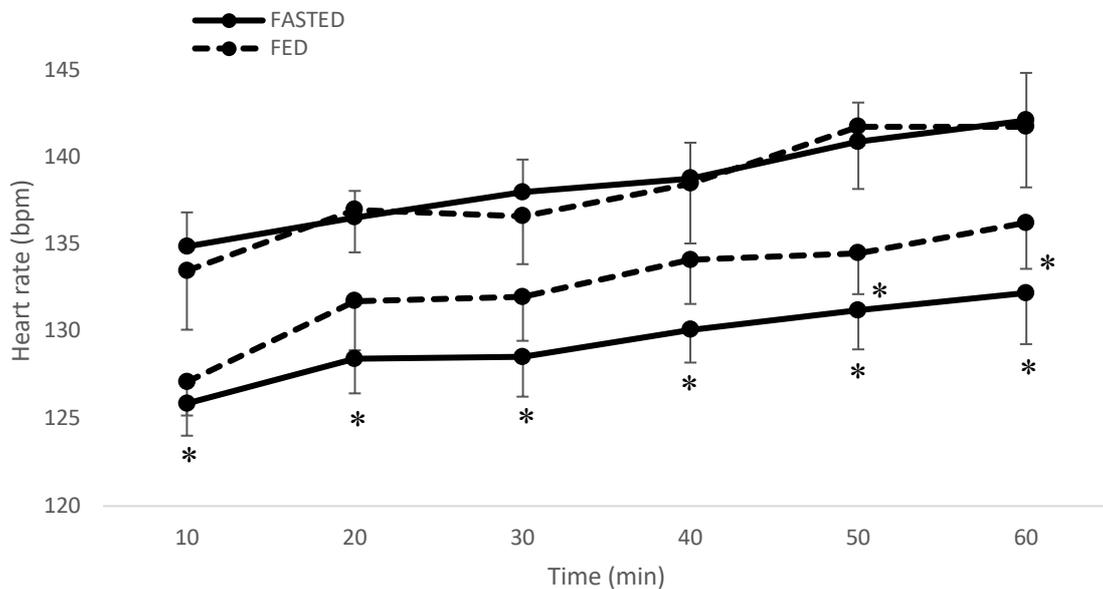


FIGURE 8. Changes in the HR during 60-minute submaximal exercise before (PRE) and after (POST) the four-week training period. Data is presented in two experimental groups: FASTED (stayed fasted after high intensity interval training (HIIT) until finishing aerobic training in the following morning) and FED (consumed food after HIIT and before aerobic training). Values are presented as mean±SE. *Significantly different from PRE ($p < 0.05$).

7.2.4 Venous blood samples

There were no differences in triglyceride and glucose concentration in the venous blood before starting the training period ($p > 0.05$). After four-weeks of training neither triglyceride nor glucose concentrations had significant changes in the blood ($p > 0.05$, Table 8). No change in hemoglobin concentration was not found after training period in either of the groups ($p > 0.05$, Table 8). Change in plasma volume after the intervention was $1.62 \pm 8.09\%$ and $-3.16 \pm 6.98\%$ in the FASTED and FED group respectively, with no significant differences between the groups ($p > 0.05$).

TABLE 8. Glucose, triglyceride and hemoglobin concentrations in venous blood before (PRE) and after (POST) four-week training period. Values are presented in two treatments: FASTED (stayed fasted after high intensity interval training (HIT) until finishing aerobic training in the following morning) and FED (consumed food after HIIT and before aerobic training).

		Glucose (mmol/l)	Triglyceride (mmol/l)	Hemoglobin (g/L)
FASTED	PRE	5.04±0.12	0.91±0.17	138.0±4.4
	POST	4.97±0.19	1.33±0.43	137.4±3.0
FED	PRE	4.82±0.17	0.84±0.10	137.0±2.8
	POST	4.97±0.23	0.79±0.11	139.3±1.9

Values are presented as mean±SE.

7.2.5 Food and training diary

There were no significant differences in total energy intake or macronutrient intake between pre-testing collection and training period collection in either of the groups ($p>0.05$, Table 7). No differences were found between the groups (Table 9). Protein intake during pre-testing was 20±2% and 18±1% in the FASTED and FED group respectively. The FASTED group had CHO intake of 44±2% and the FED group had 45±2% during pre-testing, whereas fat intake was 31±3% and 32±2% respectively. During the training period collection protein intake was 19±1% and 18±1%, CHO intake was 45±1% and 47±3% and fat intake was 31±1% and 31±2% in the FASTED and FED group respectively.

TABLE 9. Average energy, protein, carbohydrate (CHO) and fat intakes related to body mass during three-day food diary recording at pre-testing (Test) and at during four-day food diary recording at training period (Training). Values are resented as FASTED (remained from eating after HIIT until finishing aerobic training in the following morning) and in FED (consumed food after HIIT and before aerobic training).

		Energy (kcal)	Protein (g/kg)	CHO (g/kg)	Fat (g/kg)
FASTED	Test	1910±110	1.4±0.1	3.2±0.4	1.0±0.1
	Training	1760±110	1.2±0.1	3.0±0.2	0.9±0.1
FED	Test	2130±210	1.3±0.1	3.4±0.4	1.1±0.1
	Training	2120±220	1.3±0.2	3.4±0.3	1.0±0.1

Values are presented in mean±SE.

Two subjects completed 88% of the training included in the research, whereas everyone else completed all the training sessions. There were no significant differences in total training amount or different types of training outside of training involved with the research between the groups (Table 10). Other sports included various of activities from bouldering to yoga.

TABLE 10. Average endurance, strength, fitness classes, other sports and total training times over the four-week training period presented in two groups: FASTED (did not consume food after HIIT or before aerobic training in the following morning) and FED (consumed food after HIIT and before aerobic training).

	Endurance (min)	Strength (min)	Fitness classes (min)	Others (min)	Total (min)
FASTED	369±88	164±59	18±12	82±36	634±102
FED	257±84	113±42	152±90	150±60	673±71

Values are presented in mean±SE.

8 DISCUSSION

The main findings in this study were that: 1) substrate oxidation was not altered after the training intervention regardless of the nutritional approach, 2) capacity to perform high intensity exercise was higher after periodised CHO availability strategy, 3) HR was lower during submaximal exercise after periodised CHO availability.

8.1 Pilot study

Our first aim was to investigate whether remaining fasted after HIIT in the evening would cause greater fat oxidation during low intensity exercise in the following morning than when eating after HIIT and before low intensity exercise. In the present study fat oxidation was greater after fasting than when consuming food. Hence, we could speculate from this result, that the HIIT was intense enough to deplete muscle glycogen stores and therefore aerobic training was performed in low CHO availability. However, since we did not measure these variables we cannot be entirely sure in what magnitude this depletion occurred. Previous study done by Bartlett et al. (2012) showed that HIIT protocol of 6x3min at 90% VO_{2max} with three-minute active recovery between the bouts depleted muscle glycogen ~30%. Also study by Cochran et al. (2010) showed that muscle glycogen stores were depleted by 30% after performing HIIT including 5x4min at 90-95% of HR reserve with two-minute rest period. Based on these we could assume that submaximal training was done with depleted glycogen content to create low CHO availability for low intensity training session.

There were no differences in the HR and RPE during the aerobic training between the two trials, which implicates that the training with low CHO availability did not seem to be any harder than when training with normal CHO availability. These results were in accordance with our hypothesis that fat oxidation was higher during submaximal exercise after performing HIIT in the previous evening and staying fasted overnight.

8.2 Training intervention.

Our second aim was to see whether this training strategy where CHO is periodised around specific training periods would enhance training adaptations in greater magnitude than when training with chronic CHO availability. Chronic training of HIIT in the evening followed by low intensity training in the following morning, improved VO_{2max} regardless of the nutritional approach which is supported by other studies looking at training with low CHO availability. Van Proeyen et al. (2011) showed increase in VO_{2max} after six weeks of training in both overnight fasted group and fed group with no differences between the groups. Another study where subjects trained either twice a day with or without glucose ingestion between the training sessions also showed increase in VO_{2max} regardless of the nutritional treatment (Morton et al. 2009). This training strategy seemed to be enough to promote changes in VO_{2max} , whereas nutritional manipulation did not seem to have any effect on it which goes against our hypothesis.

Effect of intervention on substrate oxidation. Training with low CHO availability have shown to improve fat oxidation in trained and untrained subjects (Hulston et al. 2010; Yeo et al. 2008). However, in the present study, no change in substrate oxidation was not observed after training period regardless of the nutritional approach which is supported by other studies looking at training with periodised CHO availability (Burke et al. 2016; Marquet et al. 2016; Marquet et al. 2015). The studies showing enhanced fat oxidation rates (Hulston et al. 2010; Yeo et al. 2008) used different approach to alter CHO availability than used in the present study. In these training was commenced twice a day and restricting CHO intake between the sessions, creating low CHO availability for the second high intensity training session.

The training-diet strategy used in the present study used three different types of approaches to vary CHO availability before, during and after specific training sessions. The idea of commencing HIIT with high CHO availability, restricting CHO intake postexercise, and performing low intensity exercise with low CHO availability in the following morning after overnight fast is thought to promote potential training benefits from three different training-diet responses. This kind of training-diet system provides high CHO availability for high

intensity exercise, provides prolonged signalling responses after exercise and enhances metabolic signalling during prolonged low intensity exercise. It has been shown that when restricting CHO feeding postexercise, p38 MAPK activation was enhanced (Cochran et al, 2010) which have a role in enhancing activation of PGC-1 α (Akimoto et al. 2005) which is thought to be master regulator of mitochondrial biogenesis (Irrcher et al. 2008). Bartlett et al. (2013) showed that p53 phosphorylation, was enhanced when CHO availability was restricted before, during and after exercise. SDH activity (Morton et al. 2009) and AMPK activity (Yeo et al. 2009; Wojtaszewski et al. 2003) has shown to be enhanced when training with low CHO availability. Research by Lane et al. (2015) looked at the effect periodised CHO availability strategy on metabolic signalling. They showed that when subjects stayed fasted overnight after exercise AMPK, p38 MAPK and phosphorylation of ACC were upregulated in the following morning. Phosphorylation of ACC was further elicited by the second steady state exercise bout as well as methylation of cytochrome c oxidase subunit 4 isoform 1 (COX4I1) was increased (Lane et al. 2015).

From these, we can see that altering CHO availability around training should in theory promote enhancement in fat metabolism. However, since there was no change in fat oxidation in either of the groups in the present study, we could speculate whether the training was not 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 certain magnitude of muscle glycogen depletion. We cannot be sure whether the HIIT training in the present study was intense enough to deplete glycogen stores for right degree. Other studies from Burke et al. (2016) and Marquet et al. (2015) did not show any effect on fat oxidation either, which might be since their subjects were highly trained athletes. Since the training period was only three weeks, there might not be significant change in fat oxidation since the athletes are so close to their genetic potential.

Effect of intervention on capacity to perform high intensity exercise. Only periodised CHO availability group had improvement in maximal speed in VO_{2max} test, running time in the supramaximal test and had higher lactate concentrations after both tests. Also, the change in lactate concentrations after VO_{2max} test was significantly higher in periodised CHO availability group than in chronic CHO availability group. This implicates that training with

periodised CHO availability enhances the capacity for high intensity exercise. We can speculate that this improvement could be due to enhanced glycolysis in the periodised CHO availability group than in chronic CHO availability group to provide more energy for high intensity exercise. The enhancement in glycolysis might be due to enhanced glycolytic enzymes or higher muscle glycogen content. It has been shown that training with low CHO availability elevates resting muscle glycogen content (Hansen et al. 2005; Yeo et al. 2008). Since these factors were not measured in this present study, future studies should investigate whether this type of training with periodised CHO availability around specific training sessions elevates resting muscle glycogen content and whether glycolytic enzymes are enhanced leading to enhanced glycolysis during high intensity exercise.

Effect of intervention on HR. In the present study, HR was lower during submaximal exercise in after periodised CHO availability, whereas no change was found in the HR in the chronic CHO availability group. This could indicate enhancement in stroke volume training with low CHO availability. Previous studies did not find any changes in HR after training in either periodised or chronic CHO availability groups after training period (Marquet et al. 2015) or showed a trend of lower HR in both groups after training, but no differences between groups (Burke et al. 2016). One possible explanation could be that stroke volume was increased due to increase in blood volume which then decreased HR. There was no significant difference between the two groups in plasma volume after the intervention and therefore increased plasma volume could not explain this lower HR in the periodised CHO group. Either there was no change in hemoglobin content, meaning that blood capacity to transport oxygen would not have decreased HR. Decrease in HR cannot be explained through improved running economy either, since oxygen consumption did not decrease after the training period during the submaximal test. However, the decreased HR might have been due to enhanced extraction of the oxygen from the blood to the muscle tissue due to increased capillarisation in the working muscles. This then would have decreased the HR since less blood is needed to deliver the same amount of oxygen needed for the muscles.

Periodised CHO availability vs. training twice a day. According to previous studies (Marquet et al. 2016, Burke et al. 2016 and Marquet et al. 2015) and this present study we could say that there is growing evidence that periodised CHO availability around specific training

sessions can be beneficial for endurance adaptations and performance improvements or at least does not seem to have any detrimental effects on endurance training adaptations and performance. All of these studies have been fairly short in durations and therefore it would be interesting to investigate what effect longer training period with periodised CHO availability would have on training adaptations. Many studies investigating training with low CHO availability have used training twice a day strategy to promote lower CHO availability for the second training session. One study that used this strategy have shown improvements in performance (Cochran et al. 2015), whereas others has failed to show improvements in performance even though fat oxidation was improved (Hulston et al. 2010; Yeo et al. 2008). Therefore, we can suggest that this periodised CHO availability strategy used in the present study might be more beneficial than training twice a day strategy when the aim is to promote changes in endurance performance.

Strengths. This present research provides some additional information of how manipulating CHO intake can affect on endurance training adaptations. This study looked at the effect of periodised availability on anaerobic capacity that previous studies has not yet investigated. It offers information on trained but not elite or highly trained athletes on training with periodised CHO availability and gives a further insight of the field in training with low CHO availability.

Limitations. Since muscle biopsies were not taken, we cannot be entirely sure how effective HIIT was to deplete muscle glycogen stores and in what magnitude and whether it was enough to promote changes in metabolic signalling. We also acknowledge the limitations when using self-reporting food diaries. Training period was only four weeks and although training periods of same length or even shorter have shown improvements in these parameters, a longer training period could have provided additional information and perhaps greater adaptations and significant differences between the two treatments. Also training outside of the research was not controlled, expect no endurance training was not allowed on the training days included in the research, which might affect on the results. These all should be taken into consideration when interpreting these results.

Conclusion. This study demonstrates that this training regime where HIIT was performed in the evening followed by steady state exercise in the following morning regardless whether it was commenced with periodised or chronic CHO availability, did not affect on substrate oxidation. Notwithstanding, training with periodised CHO availability enhances the capacity to perform high intensity exercise and seems to promote other endurance training adaptations. Future studies are needed to investigate the possible role of intramuscular glycogen stores and enhanced glycolytic enzymes to improve high intensity exercise capacity shown in this present study. The present findings provide evidences to indicate that regular training with periodised CHO availability is a useful strategy to stimulate physiological adaptations that may eventually contribute to improve endurance performance.

8.3 Practical applications

According to this present study, this training-diet strategy can be integrated into endurance training program. It does not hinder any endurance training adaptations, it enhances the capacity to perform high intensity exercise and seems to promote other beneficial training adaptations. It is important to remember to ensure that CHO availability is high during high intensity training and competition to providing enough fuel that training intensity does not decrease, and performance is not affected.

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