DOES CARBOHYDRATE FEEDING DURING EXERCISE INFLUENCE ENDURANCE PERFORMANCE AND WHOLE-BODY METABOLIC PERTURBATIONS WHEN EXERCISE IS COMMENCED WITH LOW CARBOHYDRATE AVAILABILITY?

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Abstract

Background - Training with low carbohydrate availability has been shown to stimulate enhanced adaptation of skeletal muscle. These metabolic adaptations, such as upregulated markers of lipid metabolism, theoretically should convey an ergogenic benefit to endurance performance. However, the caveat to this finding is a reduction in attainable training intensity. One way to combat this limitation appears to be to consume carbohydrates during the exercise, however, traditionally this has been shown to blunt the beneficial metabolic response elicited from training low. It is possible that delaying the feeding of carbohydrate until after the start of exercise might minimise the metabolic disturbance whilst also conferring an ergogenic advantage. Therefore, the purpose of this study was to investigate the potential of a delayed carbohydrate feeding strategy in the glycogen depleted state as a viable method of maintaining a favourable metabolic response to exercise whilst also improving endurance performance.

Methods – Eight endurance trained cyclists underwent a 2-day endurance cycling protocol in three different nutritional conditions; placebo, high carbohydrate availability with carbohydrate feeding or low carbohydrate availability with carbohydrate feeding during exercise. The protocol consisted of a glycogen depletion on day 1, followed by a 6-hour refeeding protocol in which participants either consumed carbohydrate (1.2g.kg.h^{-1}) or a placebo dependent on the condition of the trial. On day 2 participants performed 1-hour of steady state cycling (50%Wmax) with gas measurements and blood samples taken at regular intervals. During the steady state bout, either carbohydrate or a placebo was consumed at 15-minute intervals dependent on condition. However, carbohydrate was fed after a delay, at 30-minutes into the exercise, in the high carbohydrate and the glycogen depleted conditions. A total of 75g of carbohydrate was consumed throughout these trials, at 15g intervals.

Immediately on completion of the steady state, subjects completed a time-trial (~40 minutes). Gas exchange measurements and blood samples were analysed to indicate the metabolic responses to each trial condition, whilst time-trial time was used as the performance measure. All data was analysed using SPSS software to identify any significant findings.

Results – There was an initial upregulation in both plasma nonesterified fatty-acid concentration and lipid oxidation in the glycogen depleted state preceding the feeding of any carbohydrate (1.41±0.34gmmol.L^{-1} and 0.68±0.15g.min^{-1} respectively) compared to the glycogen replete condition (0.97±0.57mmol.L^{-1} and 0.51±0.30g.min^{-1} respectively). However, when carbohydrate was fed 30-minutes after the onset of exercise, plasma non-esterified fatty acid concentration and lipid oxidation were significantly suppressed in the low glycogen condition (0.61± 0.25mmol.L^{-1} and 0.76± 0.13g.min^{-1} respectively, $P < 0.05$), to a similar level as in the normal glycogen condition (0.58±0.55mmol.L^{-1} and 0.62±0.29g.min^{-1}) . Furthermore, there was no observed ergogenic benefit to endurance cycling performance in a time-trial when carbohydrates were ingested in the glycogen depleted state (44.05±7.68 minutes), compared to placebo (47.74±9.73 minutes) nor was there a decrement as compared to a condition of high carbohydrate availability (42.15±8.58 minutes, $P = 0.22$).

Conclusions – The ingestion of carbohydrates during exercise in the glycogen depleted state did not convey a metabolic advantage compared to the replete glycogen condition or any performance advantage in a cycling time-trial as compared to a placebo. However, there appears to be no negative impact of this strategy in comparison to performance in the fully replete state. Therefore, further research must be undertaken in order to explore the potential of this training strategy as a tool for improving both the metabolic response to exercise and benefits to performance.

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Abbreviations

AMP, Adenosine monophosphate; AMPK, AMP-activated protein kinase; ANOVA, Analysis of variance; β-HAD, Beta-hydroxyacyl-CoA dehydrogenase; CAT, catecholamine; CD36, Cluster of differentiation-36; CHO, Carbohydrate; ChREBP, Carbohydrate-response element binding protein; CPT1, Carnitine palmitoyl transferase 1; CS, Citrate synthase; Dep-CHO, glycogen depleted with carbohydrate in exercise; Dep-PLA, glycogen depleted with placebo in exercise; EDTA, Ethylenediaminetetraacetic acid; EE, Energy expenditure; F, Fructose; FFA, Free fatty acid; FOXO1, Forkhead box protein-O1; HR, Heart rate; HRmax, maximum heart rate; HSL, Hormone sensitive lipase; IMCL, Intramyocellular lipid; IMTG, Intramuscular triglyceride; LPL, Lipoprotein lipase; MAL, Maltodextrin; mRNA, messenger ribonucleic acid; NEFA, Non-esterified fatty acid; p-ACC, Phosphorylated acetyl-CoA carboxylase; p38MAPK, p38 Mitogen-activated protein kinase; PDK4, Pyruvate dehydrogenase kinase-4; PGC-1α, Peroxisome proliferator activated receptor gamma coactivator-1; PLA, Placebo; Rep-CHO, glycogen replete with carbohydrate in exercise; RER, Respiratory exchange ratio; RPE, Rate of perceived exertion; rpm, Revolutions per minute (cadence); RQ, Respiratory quotient; SD, Standard deviation; SS, Steady state; TT, Time-trial; TTE, Time-to-exhaustion; UCP3, Uncoupling protein 3; $\dot{V}CO_2$, Carbon dioxide output; $\dot{V}O_2$, Oxygen uptake; $\dot{V}O_2max$, Maximal aerobic capacity; W, Watts (power output); $W.kg^{-1}$, Watts per kilogram; Wmax, Maximum power output.

1.0 Introduction

1.1 Background – Carbohydrate and glycogen: a substrate for physical exercise

Carbohydrates (CHO) are arguably the most critical macronutrient for moderate and high intensity exercise due to their role as a rapidly oxidisable substrate (Krogh and Lindhard, 1920). CHO is available as an exogenous fuel from a variety of dietary sources. Once consumed, CHO is metabolised and transported around the body in the form of glucose in the blood. Blood glucose is available as an immediate fuel source to the numerous sites within the body that require it, predominantly the skeletal muscle and brain. Additionally, humans have the capacity to store a finite amount of CHO in the form of the polysaccharide, glycogen. Glycogen was first discovered in 1855 as the primary storage form for CHO in humans (Bernard, 1855). It is stored at a number of sites within the body, with the majority existing in the skeletal muscle (Bergstrom and Hultman, 1966; Bergstrom et al., 1967) and liver (Cori, 1926; Van der Vies, 1954; Hultman and Nilsson, 1971). However, glycogen is also stored in more modest quantities in the brain (Cataldo and Broadwell, 1986; Brown and Ransom, 2007), kidneys (Meyer and Gerich, 2000) and adipose tissue (Rigden et al., 1990). The glycogen store within the skeletal muscles is considered the most important store for physical performance, as glycogen can be rapidly broken down and the CHO oxidised locally in order to provide energy. Bergström and Hultman (1967) were the first to report a direct linear relationship between muscle glycogen depletion and the development of fatigue during intense endurance exercise. A finding which further emphasises the importance of high levels of intramuscular glycogen storage prior to commencing a bout of endurance exercise.

The liver is also a very important storage site for glycogen and is fundamentally a reserve store for when muscle glycogen stores approach depletion. The production of glucose from the liver increases as continuous exercise duration progresses, as an additional fuel source for the working skeletal muscle (Bergstrom and Hultman, 1967). Additionally, hepatic glycogenolysis contributes significantly in the prevention of hypoglycaemia and therefore should enable athletes to perform more prolonged exercise bouts by delaying the onset of fatigue (Decombaz et al., 2011). It therefore transpires that, without a continuous supply of glucose from circulating and endogenous CHO stores, the skeletal muscle would be unable to sustain physical work.

Resulting from the evidence to show the significant importance of CHO availability in relation to physical exercise, a number of further studies have been performed with the aim of increasing glycogen storage or at least ensuring adequate CHO availability for exercise. The evidence gained from these studies has culminated in the generation of sports nutrition guidelines. These dietary guidelines are unanimous in their recommendation of the practice of ensuring high CHO availability; for routine training the recommended intake is 5-7g.kg.d^{-1} and this should be increased up to 7-10g.kg.d^{-1} during periods of intensified training (Burke et al., 2001). However, it is important to note that these guidelines are somewhat flexible and therefore differ dependent on the energy requirements of individual athletes. These requirements are regulated by the training volume and intensity that they undertake. As well as ensuring that athletes are supporting their training by consuming adequate CHO in their daily diet, there is much evidence to suggest that further performance benefits can be gained from feeding CHO during exercise.

1.2 Carbohydrate feeding during exercise

CHO and fat are the primary substrates for skeletal muscle in endurance exercise, the contribution of each respective substrate is highly dependent on the intensity of the exercise being undertaken, as well as the duration of the exercise bout (van Loon et al., 1999). High CHO availability to the muscle and central nervous system is essential to moderate-to-high intensity exercise performance (Bergstrom and Hultman, 1967; Tsintzas and Williams, 1998). As a result of physical exercise, muscle glycogen becomes increasingly depleted and consequently the reliance on blood glucose as an oxidisable substrate increases in prolonged exercise which, if not supplemented, eventually leads to hypoglycaemia and fatigue (Coggan and Coyle, 1988). Therefore, it transpires that during exercise where endogenous stores of glycogen are exceeded, it is necessary that exogenous CHO is supplemented to prevent limitations to performance (Burke et al., 2011). The metabolic and performance effects of CHO ingestion during exercise are well documented within the literature with the ergogenic effect of CHO feeding on exercise performance first documented in 1924 (Levine et al., 1924), a finding that was later corroborated by Christensen and Hansen (1939). These early studies have prompted a mass of further research into the relationship between CHO availability and exercise performance. Their findings have been repeatedly replicated and built upon in the literature over the years following.

A study conducted in 1983 investigated the effects of CHO feeding during a high intensity cycling bout compared to a placebo (PLA) trial. In this investigation, blood glucose in the CHO trial was maintained consistently above the pre-exercise concentration throughout the exercise bout. In the PLA condition, blood glucose concentration began to decrease after 60-minutes of exercise. These findings are reflected in the endurance capacity outcomes; in

the CHO trial, time to fatigue was significantly increased compared to the PLA trial. This study therefore provides evidence that frequent feeding of CHO during high intensity (70-80% $\dot{V}O_2max$) exercise can delay the onset of fatigue in trained subjects. The proposed mechanism for this outcome was that the fatigue caused by exercise could be delayed, not by offsetting hypoglycaemia but rather by slowing muscle glycogen depletion via increased blood glucose oxidation (Coyle et al., 1983). The finding of improved endurance capacity following the consumption of CHO during exercise has been further investigated, and the literature demonstrates conclusively that this finding is not an anomaly. Multiple subsequent studies have since proven CHO supplementation to augment performance in high intensity endurance exercise (Ivy et al., 1983; Coyle et al., 1986; Wright, Sherman and Dernbach, 1991; Tsintzas et al., 1993; Langenfeld et al., 1994; Tsintzas et al., 1996). Additionally, there is supporting evidence that the delayed fatigue resulting from CHO feeding occurred as a result of a reduced rate of muscle glycogen depletion due to increased exogenous CHO oxidation (Bergstrom and Hultman, 1967; Bjorkman et al., 1984; Hargreaves et al., 1984; Erickson et al., 1987; Tsintzas et al., 1995; Tsintzas et al., 1996; Tsintzas and Williams, 1998; Stellingwerff et al., 2007)

Contrary to the reports of muscle glycogen sparing in the literature, a study by Bosch, Dennis and Noakes (1994) was the first to conclude that CHO ingestion during exercise lead to the sparing of liver, but not muscle, glycogen compared to ingestion of PLA. They concluded that this was, in fact, due to the delayed onset of hypoglycaemia therefore eliminating the stimulation of liver glycogenolysis. The same study also indicated that the ingested CHO lead to increased oxidation of blood glucose as a result of an increased appearance of glucose in the blood, thus reducing the requirement for endogenous CHO oxidation and prolonging performance. This finding does not stand alone, multiple studies

have investigated the effect of CHO ingestion during exercise on substrate utilisation and many have found that despite differences in blood glucose concentrations, muscle glycogen utilisation is unchanged (Fielding et al., 1985; Coyle et al., 1986; Flynn et al., 1987; Hargreaves and Briggs, 1988; Mitchell et al., 1989). There is therefore, ambiguity surrounding the precise mechanism responsible for the postponement of fatigue resulting from CHO ingestion during exercise (Newell, Wallis and Galloway, 2014). The current perspective is that this performance effect is the result of synergy of a range of mechanisms including; prevention of reduced blood glucose concentration (Coyle et al., 1983; Coyle et al., 1986), increased exogenous CHO oxidation rate (Bosch, Dennis and Noakes, 1994; Smith et al., 2010) and, to some extent, the reduced degradation of endogenous muscle and liver glycogen (Jeukendrup et al., 1999).

Of importance with regard to the beneficial performance effects of ingesting CHO during exercise, is the time-point at which the CHO is fed during the exercise. Tabata, Atomi and Miyashita (1984) demonstrated that when CHO was fed at the point of fatigue and exercise was continued, blood glucose concentration continued to fall. This finding is suggestive that after fatigue has occurred the ingestion of CHO cannot rescue performance as the rate of appearance of glucose in the blood is too slow to meet the demands of the working skeletal muscles. This finding prompted further research into the optimal timing of CHO ingestion during exercise in order to delay fatigue. Coggan and Coyle (1988) subsequently investigated the latest point in exercise at which CHO could be fed and used to maintain performance before the onset of fatigue. They found that the ingestion of a CHO drink ~30-minutes prior to the occurrence of fatigue led to a sharp increase in blood glucose concentration compared to PLA, this caused a similar increase in respiratory exchange ratio

~15-30-minutes after the initial increase in blood glucose concentration. The most profound finding from this study was that time to fatigue during cycling exercise at 70% $\dot{V}O_2max$ was on average 21% longer when CHO was fed late in the bout compared to PLA. Thus, the results from the study indicate that a CHO ingestion late in exercise can still provide a sufficient blood glucose concentration to increase exogenous CHO oxidation and thus delay the onset of hypoglycaemia and fatigue.

It is clear from the existing literature that CHO feeding during exercise is an unequivocal strategy for delaying the onset of fatigue and thus enhancing endurance performance in moderate-to-high intensity exercise. This finding has prompted further research that has focused on uncovering various strategies to exploit the advantages that this nutritional method confers to performance. One such development has been the emergence of the practice of 'carbohydrate periodisation'.

1.3 Carbohydrate and glycogen – more than a store

Despite the overwhelming evidence in the literature to support the dietary practice of maintaining high CHO availability for both training and competition, surprisingly few athletes consume these very high CHO diets in the real world, as the recommended intakes to support their training are often unobtainable (Burke et al., 2001). Additionally, whilst the adaptive response to exercise is largely multifactorial, it has long been acknowledged that training adaptations can be either amplified or inhibited by nutritional intervention. As CHO is the main substrate for physical exercise, it is not surprising that by manipulating its intake one can in turn modify the training response (Jeukendrup, 2017). The molecular pathways that contribute to the adaptive remodelling of skeletal muscles are stimulated in exercise,

however these pathways are also sensitive to nutrients. This therefore raises the possibility that nutritional strategies may be used to manipulate the magnitude of the adaptive response to exercise, as well as the exercise performance itself (Close et al., 2016).

Consequently, evidence has emerged for a new nutritional method that might optimise both metabolic and performance outcomes. This novel method has been coined "nutritional periodisation", which can be defined as the strategic implementation of training and nutrition in combination, or solely nutrition, with the primary aim of obtaining adaptations that are facilitative of exercise performance (Jeukendrup, 2017). In contrast to the traditional approach of optimising CHO availability, nutritional periodisation advocates training with low CHO availability in strategically selected training sessions (Bartlett, Hawley and Morton, 2015). Whilst the exact mechanisms responsible remain unclear, the basis for this approach stems from evidence that the acute molecular response appears to be augmented in conditions of low CHO availability (Yeo et al., 2008; Burke et al., 2010; Philp et al., 2013; Lane et al., 2015). These molecular adaptations appear to be primarily stimulated by the exercise-induced reductions in muscle glycogen content, increased circulating non-esterified fatty acids (NEFA) and catecholamines (CATs) (Philp, Hargreaves and Baar, 2012). Post-exercise consumption of CHO has been shown to suppress these metabolic triggers and therefore might be responsible for attenuation of post-exercise adaptations (Akerstrom et al., 2006).

It has been demonstrated that 'training low' exerts a potent effect on cellular signalling and gene expression responses to acute exercise, which in turn modulates endurance training adaptations (Close et al., 2016). Much of this evidence centres on the fact that, when exercise is performed in a state of glycogen depletion, transcription factors with glycogen-binding domains are released. These associate with different targeting proteins to confer metabolic adaptations (Burke, 2010). There is evidence that substrate availability,

specifically CHO availability, can influence metabolism (Wojtaszewski et al., 2002; Cluberton et al., 2005; Pilegaard et al., 2005). Collectively, this evidence shows that the transcription, messenger ribonucleic acid (mRNA) content and expression of multiple metabolic genes are upregulated in conditions where CHO availability remains low after exercise. Pilegaard et al. (2005) found that low CHO availability in the recovery period after exercise lead to prolonged activation of pyruvate dehydrogenase kinase-4 (PDK4), uncoupling protein-3 (UCP3), lipoprotein lipase (LPL), carnitine palmitoyl transferase-1 (CPT1), cluster of differentiation-36 (CD36) and forkhead box protein-O1 (FOXO1), whereas high CHO availability lead to a reversal of the exercise-induced activation of these genes. Similarly, Cluberton et al. (2005) were able to demonstrate that substrate availability could modulate changes in metabolic gene expression as a result of altered transcription and mRNA content. They showed that the ingestion of glucose in recovery from exercise caused suppression of the exercise-induced upregulation of metabolic gene expression. This indicates that in order to sustain the effect of exercise on gene expression and thus enhance training adaptation, CHO should be withheld. The resulting increase in metabolic stress acts as a strong stimulus for adaptation. 5-AMP-activated protein kinase (AMPK) has received much attention in the area of training adaptation. Wojtaszewski et al. (2002) investigated the relationship between AMPK activity and muscle glycogen content. They found evidence to show that glycogen content can directly regulate the activity of AMPK in vivo, this is a significant finding when considering the metabolic effect of low CHO availability on training adaptation due to the role of AMPK as a ‘metabolic switch’. This study indicates that low glycogen content leads to an upregulation of AMPK expression and activity, resulting in a reduced reliance on glycogen metabolism and increased liberation and oxidation of free fatty acids (FFAs) from intramuscular triglyceride (IMTG) and adipose.

The increased cellular stress associated with reduced CHO availability has been shown to influence the activity of several molecular pathways involved in training adaptation. These include increased phosphorylation and activity of AMPK, p38 mitogen-activated protein kinase (p38 MAPK) and peroxisome proliferator activated receptor gamma coactivator-1 (PGC-1α). All of which result in the co-activation of transcription factors which subsequently lead to the expression of mitochondrial proteins, thus stimulating increased mitochondrial biogenesis (Bartlett, Hawley and Morton, 2015). Additionally, the increase in circulating CATs resulting from low glycogen content leads to the increased activity of hormone sensitive lipase (HSL) which causes increased liberation and oxidation of FFA from both adipose tissue and IMTG (Kiens, 2006). This finding is indicative of an increased dependence on lipid metabolism as a result of low muscle glycogen stores at the onset of exercise. CHO response element-binding-protein (ChREBP) has been shown to be suppressed when glycogen content is reduced. ChREBP is thought to be important in the regulation of glycolysis, therefore this suggests that its suppression is in part responsible for the increase in lipid oxidation that occurs in conditions of glycogen depletion (Ishii et al., 2004). The discovery that CHO restriction during both exercise and recovery can upregulate these signalling pathways that are key in the regulation of training adaptation has led to speculation that training with low CHO availability can improve endurance performance in athletes (Pilegaard et al., 2005; Psilander et al., 2013). This is because the molecular adaptations observed as a result of training with low CHO availability indicate that there will be an increase in oxidative capacity during exercise (Philp, Hargreaves and Baar, 2013).

1.4 Manipulating training adaptations in practice

As a result of the relatively recent discovery that training in a glycogen depleted state can lead to supposedly beneficial metabolic adaptations and potentially improvements in performance, a number of different strategies for implementing the train low concept have been investigated. These include models such as; training twice-a-day (Hansen et al., 2005; Yeo et al., 2008; Hulston at al., 2010), withholding carbohydrate in the post-exercise period (Pilegaard et al., 2005), training in a fasted state (Van Proeyen et al., 2011), protein-only sessions and sleep low strategies (Lane et al., 2015; Marquet et al., 2016a; Marquet et al., 2016b).

The most simplistic protocols used in research investigating the effect of low CHO availability on performance and metabolism are either withholding CHO feeding during a recovery period and training in the fasted state. Pilegaard et al. (2005) investigated the effect of withholding CHO in the recovery period from high intensity endurance cycling exercise on the regulation of metabolic genes. They demonstrated that this protocol lead to no restoration in muscle glycogen concentration and increased levels of plasma FFAs. This study provided clear evidence that the feeding of CHO in the recovery period leads to suppression of the enhanced transcriptional response of several metabolic genes involved in training adaptation to exercise. Van Proeyen et al. (2011) utilised a training-in-the-fasted-state model and demonstrated that whilst performance outcomes were improved to the same extent as training with high CHO availability, metabolically there were significant differences in the response to exercise. It was found that during exercise in the fasted state the breakdown of intramyocellular lipids (IMCLs) was upregulated, as was the maximal rate of fat oxidation. This metabolic effect however did not subsequently lead to any improvement in endurance

performance, a finding that may in part be explained methodologically by the acute nature of the training intervention period. Therefore, it is likely that if a similar protocol were carried out chronically, the metabolic advantages observed may also lead to an enhancement in endurance performance.

The most commonly used model in research is the "twice-every-second-day" training protocol; two training sessions in a day, with the second performed in a state of low glycogen availability (Hansen et al., 2005). The first study to utilise a single leg training model in order to directly compare the effect of two training protocols on the skeletal muscle adaptation in the quadriceps of the same individual found that the train low protocol lead to an increased post-training time to fatigue, greater resting muscle glycogen concentration and enhanced citrate synthase (CS) activity compared to the training once daily protocol (Hansen et al, 2005). It is important to note that the finding of increased time to fatigue did not result from the increased resting muscle glycogen content. Therefore, this pioneering study provides adequate evidence to justify further investigation into the efficacy of the use of train low strategies to enhance training adaptation and potentially performance. However, training in a condition of low muscle glycogen concentration leads to a much-reduced training intensity, this is counterintuitive for the elite athlete who relies on high-intensity training in order to gain a competitive edge and improve their performance (Hawley and Morton, 2014). Additionally, this study was carried out with a population of untrained individuals and therefore it is difficult to predict whether or not any beneficial effects would occur in already well-trained athletes.

A pair of studies conducted by Yeo et al. (2008; 2010), have investigated both the chronic and acute effects of endurance training with low CHO availability on training adaptation and performance. The first of these studies (Yeo et al., 2008) adopted a training

twice-every-second-day approach compared to a once-daily approach, both training programmes were adopted for 3-weeks. Unlike the study of Hansen et al. (2005), this study was carried out with a population of well-trained individuals and utilised a training mode that is more generalisable to the routine of elite athletes. The study found that multiple markers of training adaptation were enhanced as a result of the chronic train low model; resting muscle glycogen content was increased, as were rates of whole-body lipid oxidation during submaximal exercise and the activity CS and β-hydroxyacyl CoA dehydrogenase (β-HAD). However, no benefit to endurance performance was observed, this was reportedly due to the reduced ability of the athletes to perform at high intensities in training thus limiting the stimulus for any significant gains in endurance. However, it is important to report that although no superior performance effect was gained from training-low twice-every-second-day, there was also no performance detriment compared to training high once-daily (Yeo et al., 2008). A further study by the same research group, conducted with the intent of elucidating the mechanisms responsible for the metabolic effects resulting from the train low strategy, found that as a consequence of performing a high intensity training session with low muscle glycogen availability, AMPK phosphorylation was significantly elevated (Yeo et al., 2010). This indicates that the muscle glycogen availability at the onset of exercise is a key regulator of AMPK phosphorylation during exercise which may in turn lead to favourable metabolic adaptations.

As a result of the significant reduction in training intensity of the training twice a day model, novel strategies had to be developed in order to attempt to negate the negative aspects of training with reduced glycogen availability. One such strategy is the “train high, sleep low” model (Lane et al., 2015), which aims to extend the period in which an athlete spends in the glycogen depleted state by delaying CHO intake overnight and as such should theoretically

enhance the acute response of several important metabolic genes with regard to training adaptation. It was found that the sleep low condition lead to increased upregulation of multiple metabolic markers with potent roles in the regulation of fat oxidation. For example, increased phosphorylation of AMPK, p38MAPK and phosphorylated acetyl-CoA carboxylase (p-ACC). Therefore, as might be expected, fat oxidation during 2-h cycling exercise was augmented. However, there was no indication of the upregulation of any of the markers of mitochondrial biogenesis, a result that has been previously demonstrated in earlier 'train low' studies (Cochran et al., 2015). This study provides evidence for the occurrence of metabolic adaptations following a sleep low protocol, however the results do not provide any insight into the effect of a sleep low strategy on the parameters of endurance performance.

More recently, the effect of the sleep low strategy on endurance performance has been studied more closely. Marquet et al., (2016a) found that athletes improved their 10-km running performance, as well as their submaximal aerobic efficiency during cycling. This study is the first to report a significant benefit of a chronically implemented train low strategy on 'real-world' athletic performance, however it provides no insight into the mechanisms that might be responsible, as limited metabolic factors were investigated. A second study was conducted in order to determine whether this model could be implemented acutely and maintain the same performance outcome (Marquet et al., 2016b). After only 1-week, cycling time-trial performance was improved to a similar magnitude as in the chronic study. It was also found that this performance increment was related to pacing strategy and self-selected power output, indicating that metabolic factors do not solely underlie the performance effects of a train low strategy. Due to the earlier finding that resting muscle glycogen concentration is increased as a result of a train low strategy (Hansen et al., 2005; Yeo et al., 2008; Yeo et al.,

2010), it can be speculated that muscle glycogen content prior to the time-trial was greater in the sleep low group thus accounting for the differences in performance. Interestingly, this study found that the observed performance improvement was not related to any of the classical metabolic perturbations associated with low CHO availability. Fat oxidation during a steady state bout was unchanged and there were no indications of upregulated lipid metabolism in the blood plasma samples. This finding highlights the apparent disconnect between the effect of train low on performance and metabolism.

Interestingly, it has been demonstrated that the feeding of CHO during exercise can confer a compensation effect when pre-exercise glycogen concentration is low. Widrick et al. (1993) investigated the effect of CHO ingestion during exercise in different conditions of glycogen concentration (high vs low) on performance in a time-trial (TT). This study provides evidence to show that the feeding of CHO in conditions of low muscle glycogen leads to a recovery of performance to a similar standard as in the high glycogen condition. Additionally, they found that respiratory exchange ratio (RER), when glycogen was low, remained unchanged even when CHO was fed to participants and was significantly lower than in the high glycogen condition. This indicates a shift in metabolism towards fat oxidation. The authors conclude that the ergogenic benefit observed resulted from an increased ability to maintain euglycaemia in the final stages of exercise, when endogenous CHO availability would otherwise be a limiting factor to performance. However, it is important to note that the glycogen concentration in the low condition of this study was between 429.1 and 471.7mmol.kg^{-1} dry weight, which in the context of contemporary papers investigating the train low approach is not especially low. A recent review states that a pre-exercise glycogen concentration of $\leq$ 300mmol.kg^{-1} dry weight appeared to be associated with optimal adaptations (Impey et al., 2018). Therefore, further research is required to elucidate whether

or not the ergogenic effect of CHO feeding observed by Widrick et al. (1993) will occur when glycogen content is critically low.

It is clear that CHO can no longer be viewed simply as a fuel source for exercise, but rather as a 'training regulator' which can be used to manipulate the training response. This is a new era for sports nutrition and can be labelled as 'targeted nutritional periodisation', the objective of which is to maximise performance and adaptation. It is essential that these strategies are carefully and strategically planned by athletes and their coaches in order to ensure that adaptation is obtained without compromising performance.

1.5 Strategies to increase workload in the face of low carbohydrate availability

Although the train low concept offers a number of preferential metabolic adaptations, these are not always translated into a performance benefit. This has been found to be largely due to the fact that training with low CHO availability leads to a decline in the attainable exercise intensity (Widrick et al., 1993; Yeo et al., 2008; Hulston et al., 2010) which can potentially reduce the overall training stimulus and thus limit performance gains. In an effort to overcome this negative performance effect, a number of strategies have been investigated in order to determine whether or not they can be used in conjunction with a train low strategy to rescue performance. These include CHO mouth rinsing and caffeine consumption (Bartlett, Hawley and Morton, 2015).

A review by Jeukendrup, Rollo and Carter (2013) reports that multiple studies investigating the effect of CHO mouth rinse on performance have provided evidence of an ergogenic effect. This effect was most pronounced in studies where participants were fasted.

A recent study has since reinforced these findings (Ataide-Silva et al., 2016), demonstrating that the practice of CHO mouth rinse lead to greater blood glucose concentrations during constant load exercise in all conditions. Additionally, this study showed that in the glycogen depleted state when CHO mouth rinse was administered, 20km TT performance and power output were increased significantly compared to PLA. The paper concluded that the potential mechanism responsible for the 'rescue' of performance in the glycogen depleted state was an augmentation of central motor drive. This evidence is indicative that CHO mouth rinsing during exercise in the glycogen depleted state may be a viable strategy of reducing the negative connotations of training low (i.e. reduced exercise intensity) whilst also maintaining the metabolic benefits.

It is widely evidenced that caffeine can be used as an ergogenic aid for performance, it has been reported to lead to greater power outputs (Ivy et al., 1979) and increased speed in simulated race conditions (MacIntosh and Wright, 1995). Much of this evidence showing the use of caffeine as ergogenic for performance has been undertaken in conditions of high CHO availability. However, there is some evidence to suggest that it can be used to partially offset the reduction in exercise intensity resulting from exercising with low muscle glycogen is the consumption of a low dose (3mg.kg^{-1}) of caffeine (Goldstein et al., 2010). An early study by Ivy et al. (1979) provides a clear indication that caffeine can be used as a method of increasing workload during exercise as a result of the increased fat oxidation, but also suggest that the effect caffeine exerts on the central nervous system also has a role to play. It is important to note that caffeine is not consistently associated with increased fat oxidation in the literature, the main mechanism thought to be responsible for its ergogenic effects is thought to be non-metabolic. Additionally, further research has demonstrated that in both the conditions of normal and low muscle glycogen content, ingestion of caffeine leads to greater

power output in cycling exercise. However, in the low glycogen condition when caffeine was fed power output could not be rescued to the same level as normal glycogen concentration (Lane et al., 2013). Despite this, caffeine consumption clearly presents a viable strategy for improving performance in the face of low CHO availability.

Both CHO mouth rinse and caffeine consumption therefore might offer a solution to the problem of a reduced training intensity associated with low muscle glycogen concentration. These strategies are especially useful as they offer an ergogenic benefit to performance without compromising the altered metabolic environment associated with the train low approach. As such the low CHO availability mechanism of adaptation will not be compromised. It is however important to note that these strategies do not fully restore performance and the long-term effects have not been investigated. Therefore, it follows that the optimal strategy for improving performance when glycogen content is low is consumption of CHO. However, this approach might seem counterintuitive as consumption of CHO is typically associated with an increase in insulin and a reduction in circulating NEFAs thus removing the metabolic stimuli for adaptation (Morton et al., 2009). Nevertheless, a study by Coggan and Coyle (1989) found that feeding CHO late in exercise but before the point of fatigue lead to an increase in plasma glucose concentration without an increase in insulin concentration or a significant reduction in plasma FFAs. Thus, indicating that delayed CHO feeding during exercise in the glycogen depleted state might be viable strategy for improving performance without altering metabolic environment or the hormonal milieu associated with low glycogen concentration.

1.6 Aims and hypothesis

Following a thorough review of the relevant literature, it is clear that training with low CHO availability can be exploited as a means of conveying a metabolic advantage i.e. upregulated lipid oxidation. However, this supposedly favourable metabolic environment has not been consistently shown to convey any particular benefit to endurance performance largely as a result of the limited capacity for physical work associated with low muscle glycogen concentration. The feeding of CHO during exercise in a condition of low glycogen concentration appears to have the potential to rescue performance, particularly when this feeding is delayed after the onset of exercise as this does not impact upon key metabolic factors that underpin the beneficial adaptive response to low CHO availability. However, this has not been directly tested, therefore this study proposes to investigate the performance and whole-body metabolic effects of 3 different conditions; low muscle glycogen with no CHO availability, low muscle glycogen with high CHO availability during exercise and normal muscle glycogen content with high CHO availability during exercise.

The aims of the present study are as follows;

1. To characterise the metabolic response to delayed CHO feeding during exercise in the glycogen depleted state.
2. To assess the effect of delayed CHO feeding in the glycogen depleted state on endurance performance.

Therefore, the primary hypothesis of the proposed study is that delayed ingestion of CHO during exercise in a state of glycogen depletion will lead to upregulated markers of lipid utilisation compared to the normal CHO replete state. The secondary hypothesis is that

performance will be improved with delayed CHO ingestion during exercise in a condition of low glycogen compared to feeding of PLA.

2.0 Methods

2.1 Participants

9 (8 male, 1 female) well-trained cyclists and triathletes were recruited for the present investigation (Table 1). One male participant dropped-out due to illness, therefore the remaining 8 healthy participants completed the study. The experimental protocol and procedures were approved by the University of Birmingham Science, Technology, Engineering and Mathematics (STEM) Ethics Committee (ERN_17-1236). Informed consent was obtained from all participants prior to testing.

Table 1. Participant characteristics.

	All (N = 8)	Males (N = 7)	Female (N = 1)
Age (y)	27±5	27±4	33
Height (cm)	174.6±7.0	174.8±7.5	173
Body mass (kg)	66.5±4.3	67.0±4.4	62.7
$\dot{V}O_{2max}$ (ml.min^{-1})	3825±488	3903±470	3279
$\dot{V}O_{2max}$ (ml.kg.min^{-1})	59.0±4.6	60.0±4.1	52.3
W_{max} (W)	342±39	351±34	283
W.kg^{-1} (W)	5.3±0.8	5.4±0.7	4.5

Values are mean ± standard deviation (SD). Abbreviations: $\dot{V}O_{2max}$ = maximum oxygen uptake, W_{max} = maximum power output, W.kg^{-1} = watts per kilogram.

2.2 Study design and research methodology

The present study used a double-blind, cross-over design and involved a total of nine visits to the laboratory (1 preliminary, 1 familiarisation and 3 experimental trials).

On the first visit to the laboratory preliminary testing was carried out. On arrival, the study protocol was thoroughly explained to the participant. Written consent (Appendix 1) and a general health questionnaire (Appendix 2) were completed before any testing was carried out. Participants were eligible to take part in this study if they were aged between 18 to 35, a non-smoker, in good general health and engaged in endurance-based exercise (e.g. cycling, running, swimming) at least 3 times per week. If these inclusion criteria were met, an incremental test to volitional exhaustion on a cycle ergometer was carried out to measure the maximal oxygen uptake ($\dot{V}O_{2max}$) and maximum power output (W_{max}). A minimum $\dot{V}O_{2max}$ value of 50mL.kg.min^{-1} and 55mL.kg.min^{-1} was required for female and male participants respectively in order for progression to the next phase of the study. Participants returned to the laboratory at a later date for the familiarisation trial in order to undertake the protocol prior to experimental trials (visits 4-9) and to ensure the participants were fully aware of the protocol. The first of three experimental trials was conducted after successful completion of the familiarisation. Subsequent trials were conducted at least 7-d apart to allow for sufficient recovery time. The order of experimental trials was randomly generated; both participant and experimenters were blinded to trial order.

2.3 Preliminary testing

Following satisfaction of the terms of the inclusion criteria, participant height (cm, Model 220, Seca, GER) and body mass (kg, Champ II, OHAUS, SWI) were measured and recorded. An incremental exercise test to volitional exhaustion on a cycle ergometer was performed on a lode bike (LodeExcalibur Sport 925900, Groningen, Netherlands). Prior to this test, the cycle ergometer was adjusted to ensure that the saddle and handlebars were in a comfortable

position for the participant. These positions were recorded and replicated during all subsequent trials.

The test was commenced at a workload of 100W, this was increased by 30W every 2-minutes until the participant could no longer maintain their cadence above 50rpm. Throughout the test breath-by-breath indirect calorimetry was recorded using a facemask (Hans Rudolph) connected to a metabolic cart (JAEGER® Vyntus CPX, CareFusion, GER) to determine rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). Heart rate (HR) was monitored continuously throughout exercise using telemetry; chest strap and watch (Polar Electro, FIN). HR and rating of perceived exertion (RPE) were recorded in the last 30-seconds of each 2-minute stage of the test. RPE was determined using the Borg Scale (Appendix 3); a measure of physical effort on a 15-grade scale from 6 (very light) to 20 (maximal exertion) (Borg, 1982).

$\dot{V}O_2max$ was assumed to be achieved at the point at which there was a plateau in oxygen consumption and the participant could no longer sustain a cadence of >50rpm. At this time-point a 30-second average of $\dot{V}O_2$ was taken and used to calculate $\dot{V}O_2max$.

Participants who achieved the required minimum $\dot{V}O_{2max}$ values (female = 50mL.kg.min^{-1}, male = 55mL.kg.min^{-1}) were eligible for further participation in the study (familiarisation and experimental trials). These participants were instructed to record a 2-day food and fluid consumption log (Appendix 4) prior to the next visit. This allowed for replication of the same diet and training volume prior to each of the experimental trials. Additionally, participants were required to abstain from exhaustive exercise for 48-h and from caffeine and alcohol for 24-h prior to the familiarisation and experimental visits. Any participants who did not achieve the required $\dot{V}O_{2max}$ were not invited to participate in further visits.

Additionally, Wmax was calculated from this test, it was determined as the power output from the last completed 2-minute stage of the test plus the time fraction spent in the next stage multiplied by 30W (Currell, Jentjens and Jeukendrup, 2006).

$$Wmax = W + {}^{n}/120 \times 30W$$

The Wmax data from the preliminary testing was used to calculate values for use in the subsequent experimental visits. The experimental protocol required calculation of 90, 80, 70 and 50%Wmax values for each participant.

2.4 Familiarisation trial

The familiarisation trial followed the identical procedure to the experimental trials with the exclusion of blood withdrawal. Following the preliminary testing visit, participants attended the laboratory at a later date at ~1300h with participants having refrained from any strenuous activity for 48-h and avoided caffeinated and alcoholic beverages on the day of the visit. Participants had also recorded their food and fluid intake prior to this visit in a 2-day weighed food diary. This intake was then replicated prior to all following experimental trials in this study. The visit took place over 2 days; day 1 was an afternoon session and day 2 a morning session the following day.

Day 1 – participants entered the laboratory at ~1300h having had their last meal at least 3-h earlier. They then completed a depletion protocol on a lode bike (Lode Excalibur Sport 925900, Groningen, Netherlands), which was set to the same saddle and handlebar

heights as in the preliminary test. The depletion ride consisted of 2-minute blocks of cycling exercise alternating between low and high intensity (see section 2.6).

Immediately post-exercise participants received the designated nutrients according to the experimental condition (see section 2.5). Water intake was available ad libitum at all times during testing and was recorded by the experimenter.

Day 2 of the familiarisation trial commenced the following morning with participants arriving at ~0700h in an overnight fasted state. On arrival, a protein gel (SIS WHEY20; 20g protein, 2g CHO, 0g fat) was consumed 45-minutes prior to the start of exercise. Day 2 consisted of 2 parts; firstly a 60-minute bout of steady-state (SS) cycling at 50%Wmax followed immediately by a TT in which a fixed amount of work was completed. Throughout the exercise protocol participants were given calorie-free flavoured drinks; during SS these were supplied at 15, 30, 45 and 60-minute time points and additionally at 1/3 and 2/3 through the TT. Substrate utilisation was monitored throughout the SS exercise using indirect breath-by-breath calorimetry with a small mouthpiece and nose clip for ~3-minutes at 11.5, 26.5, 41.5 and 56.5-minute time points. RPE was measured at 15-minute intervals during SS and HR was continuously monitored using telemetry and recorded every 15-minutes in SS and at 1/3 and 2/3 completion of the TT.

2.5 Nutritional interventions

This study implemented 3 different nutritional interventions in a randomised, cross-over manner. The order of conditions was double-blinded, therefore all drinks and jelly consumed by the participants were prepared by an external investigator. Participants were allowed to

drink water ad libitum throughout all 3 trial conditions, the volume of water consumed was recorded.

Table 2. Carbohydrate provision on day 1 and day 2 of the experimental trials in each nutritional intervention condition.

	Day 1		Day 2					
	<30-minutes post-exercise	6h refeeding period	15-minutes	30-minutes	45-minutes	60-minutes	1/3 TT	2/3 TT
Rep-CHO	SIS Whey Protein Gel	1.2g.kg.h^{-1}	0g	15g	15g	15g	15g	15g
Dep-CHO	SIS Whey Protein Gel	0g	0g	15g	15g	15g	15g	15g
Dep-PLA	SIS Whey Protein Gel	0g	0g	0g	0g	0g	0g	0g

Abbreviations: TT = time-trial.

A) Glycogen depletion with CHO intake during exercise on Day 2 (Dep-CHO) Following completion of the glycogen depletion on Day 1, participants were provided with low-calorie flavoured drinks (Robinson's No Added Sugar Orange Squash) and jelly (Hartley's Sugar Free Strawberry Jelly). The first dose was provided immediately after exercise and subsequently at 30-minute intervals over the following 6-h. Participants consumed the first dose in the laboratory, they were then provided with a dosage timescale and allowed to consume the rest of the drinks and jelly at home. Additionally, a protein gel was consumed in the first 30-minutes post-exercise.

On Day 2, on entering the laboratory participants consumed a protein gel. Participants later received a total of 1200ml flavoured drink (Robinson's No Added Sugar Orange Squash) with 75g of added maltodextrin (MAL) during exercise. The drinks were 200ml in

volume and were provided at 15, 30, 45 and 60-minutes in the SS and at 1/3 and 2/3 completion of the TT work. The first feeding at 15-minutes was PLA and therefore contained no CHO. The subsequent drinks each contained 15g of MAL. This dosage of CHO during exercise has been reported to significantly improve performance in cycling exercise compared to ingestion of PLA, additionally it was demonstrated that any further increase in the rate of intake per hour led to no significant benefits in performance (Newell et al., 2018).

B) Glycogen depletion with PLA intake during exercise on Day 2 (Dep-PLA)

This condition follows the same procedure as in condition A for the first 6-h post exercise, with low-calorie flavoured squash and jelly provided every 30-minutes and a protein gel consumed in the first 30-minutes post-exercise.

On Day 2 of the trial, participants consumed a protein gel on arrival to the laboratory. The participants then received the same volume of flavoured drink as in condition A (1200ml), but without any added MAL. Thus, 200ml of flavoured low-calorie drink was consumed at 15, 30, 45 and 60-minutes of SS, and at 1/3 and 2/3 completion of the TT.

C) Glycogen repletion on Day 1 with CHO intake during exercise on Day 2 (Rep-CHO)

Following the glycogen depletion on Day 1, participants were provided with 1.2g.kg.h^{-1} of CHO (2:1 MAL:fructose [F]) over the 6-h post-exercise period. This is in line with current sport nutrition recommendations for athletes in order to fully replenish their glycogen stores post-exercise (Burke, van Loon and Hawley, 2016). This dosage was appropriate for the limited recovery time of participants as it is advised that 1.0-1.2g.kg.h^{-1} of CHO be consumed in the first hours of the post-exercise recovery period (van Loon et al., 2000; Beelen et al., 2010; Burke et al., 2011). A higher dosage of CHO would have had no further effect on the

rate of glycogen resynthesis. It has been reported that CHO ingestion rates of >1.2g.kg.h^{-1} do not lead to any further increase in resynthesis rates (Howarth et al., 2009; Beelen et al., 2010). The inclusion of F as well as MAL in the recovery feedings is due to the need to recover liver glycogen concentration as well as muscle stores. The combined feeding of F with MAL has been shown to double the rate of liver glycogen repletion whilst also restoring intramuscular glycogen concentration (Decombaz et al., 2011; Fuchs et al., 2016).

This CHO was added to low-calorie squash and jelly and provided immediately post-exercise and every 30-minutes thereafter for 6-h. As in conditions A and B, the first feeding occurred in the laboratory, after which the participant consumed the CHO enriched jelly and squash at home. As in conditions A and B, in the first 30-minutes post-exercise the participant consumed a protein gel.

On Day 2, participants consumed a protein gel on entering the laboratory. Participants then received 200ml of flavoured drink at 15, 30, 45 and 60-minutes during SS and at 1/3 and 2/3 completion of the TT. The first drink consumed at 15-minutes into the SS contained no CHO, thereafter all drinks contained 15g MAL.

The inclusion of protein gels as a post-exercise and pre-exercise ‘meal’ in this study in addition to the nutritional interventions administered, was a step taken to ensure comfort of the participant as a previous study utilising a similar protocol reported ‘hunger pains’ without this supplement (Currell, Jentjens and Jeukendrup, 2006). Additionally, this made it more difficult for participants to guess which experimental condition they were in due to differing levels of hunger.

2.6 Experimental trials

Following the familiarisation trial, participants attended the laboratory at ~1300h having refrained from strenuous activity for 48-h and avoided caffeine and alcohol for 24-h. Participants were responsible for ensuring that they had consumed the same diet as they had prior to the familiarisation for 2-days prior to the experimental visit. The protocol for the experimental trials is depicted in figure 1.

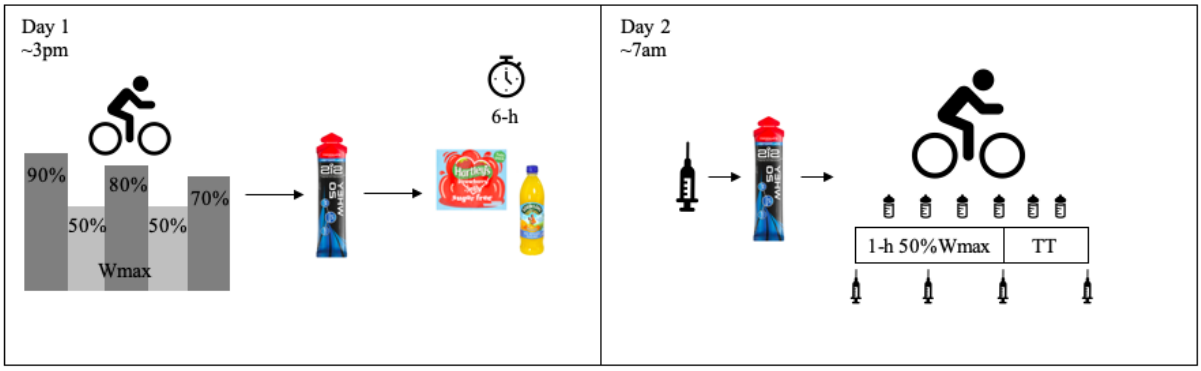

Figure 1. Protocol schematic for the experimental trials.

On arrival, participant weight was recorded. Participants firstly performed a 5-minute warm-up at 50%Wmax on the lode bike. Immediately on completion of the warm-up, participants began the glycogen depletion which consisted of 2-minute blocks of cycling, alternating between low (50%Wmax) and high (90%Wmax) intensity bouts. When cycling at 90%Wmax became intolerable (i.e. the full 2-minute period could not be completed), the high intensity block was reduced to 80%Wmax and subsequently to 70%Wmax when 80%Wmax could no longer be sustained. The point at which 70%Wmax became intolerable marked the termination of exercise (Kuipers et al., 1989). This protocol has been utilised extensively in the literature as a means of eliciting muscle glycogen depletion in trained subjects and has

consistently been demonstrated as a reliable method for reducing intramuscular glycogen levels (Van Hall et al., 2000; Van Loon et al., 2000; Jentjens et al., 2001; Wallis et al., 2008). Immediately following the cessation of exercise, participants were first weighed and then provided with a commercially available protein gel (SIS WHEY20) and flavoured drinks and jelly in accordance with the designated nutritional condition of the trial (A/B/C) for the subsequent 6-h. Both participant and investigator were blinded to the condition.

Participants reported to the laboratory the following morning at ~0700h for Day 2 of the trial. On arrival their weight was recorded, however they were not permitted to see their current weight in order to prevent any potential feedback of the condition they were in. They were then fitted with an antecubital venous cannula and a resting blood sample of 10ml was obtained. Immediately following this the participant consumed an SIS WHEY20 protein gel.

~45-minutes after the consumption of the protein gel, another ~10ml blood sample was taken and exercise was commenced. Day 2 of the trial consisted of 2-parts (figure 2). Firstly 1-h of SS at 50%Wmax, during which 10ml blood samples were taken at 30 and 60-minute time points and flavoured drinks (PLA or CHO dependent on trial condition) were consumed regularly (15, 30, 45 and 60-minutes). In both trials the drink consumed at 15-minutes was PLA, regardless of condition, the remaining drinks in the CHO trial (30, 45 and 60-minutes) all contained CHO. Additionally, substrate utilisation was monitored regularly throughout using indirect breath-by-breath calorimetry. A small mouthpiece and nose clip were worn for 3-minutes at 11.5, 26.5, 41.5 and 56.5-minute time points. During the SS phase of the trial, exertional measures (RPE and HR) were recorded every 15-minutes. The cycle ergometer was set to cadence independent mode and cadence was self-selected throughout. Immediately following the completion of 1-h SS, the cycle ergometer was set to linear mode, by which the workload increases proportionally to the cadence according to the formula:

$$W = L(rpm)^2$$

Where *rpm* is cadence and *L* is a linear factor (Currell, Jentjens and Jeukendrup, 2006). The linear factor for each participant was calculated so that at a cadence of 80rpm the power output of the work being performed would elicit ~65% of their $\dot{V}O_2max$. Participants then began a TT with a fixed amount of work to be completed. The total amount of work to be completed in the TT was calculated according to the formula from Currell, Jentjens and Jeukendrup (2006):

$$Total\ work = 0.65Wmax \times 2400J$$

The cycle ergometer was connected to a computer with the TT software, which recorded power output, cadence and work completed throughout the trial. The participants were informed of their progress by the experimenter after every 10% of the total work was complete but received no other feedback. Performance results were withheld until completion of the study. The removal of external feedback during the TT, except for knowledge of the work completed, was intended to minimise extrinsic motivation in order to ensure that there were limited factors affecting variability of performance (Currell and Jeukendrup, 2008).

During the TT participants received flavoured drinks (PLA or CHO) after 1/3 and 2/3 of the work had been completed. Cadence was self-selected however participants received no feedback on their cadence during the TT. Upon completion, another 10ml blood sample was taken. HR was noted at 1/3 and 2/3 completion of the TT. On completion of the TT, participant weight was recorded.

During the SS phase participants were permitted to listen to music or watch television, however this was standardised between the 3 trials in order to control motivational influences. As soon as the TT was commenced this was no longer permitted.

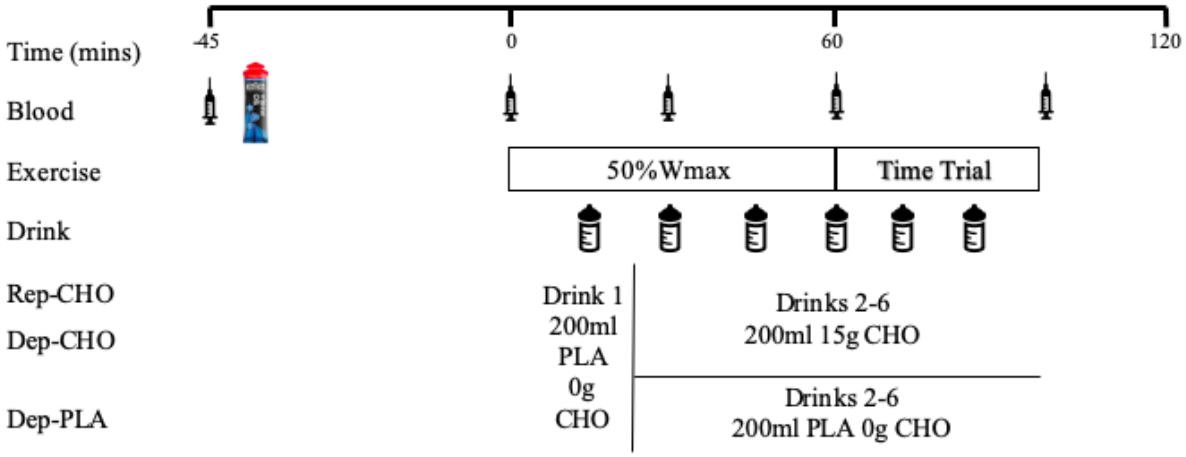

Figure 2. Schematic of the protocol design for experimental trial visit 2.

Experimental trials 1 and 2 were separated by 16±9 days and trials 2 and 3 were separated by 19±10 days. Trial conditions were randomised and double-blinded. All flavoured drinks and jelly were prepared by an external investigator. Additionally, the influence of environmental conditions was kept to a minimum. Temperature was controlled at 20.0±0.8ºC and humidity at 49.8±6.8%. Floor standing fans were angled behind participants during cycling exercise to ensure adequate heat loss by convection.

Traditionally, in performance research, a time-to-exhaustion (TTE) protocol was historically used in order to detect differences between experimental interventions. However, the issue with this method of testing is that rather than assessing performance as the outcome measure, it tests exercise capacity (Williams et al., 1990). It has subsequently been reported that TTE protocols have poor reliability when compared to the use of TT (Jeukendrup et al., 1996). This finding has prompted an increase in the use of TT protocols in performance

research. Two previous studies have investigated performance in the glycogen depleted state, with similar protocols to the present study. These studies found the coefficient of variance to be similar; 3.7% (Currell, Jentjens and Jeukendrup, 2006) and 3.5-5.7% (Widrick et al., 1993). A finding of significance, as this indicates that reducing muscle glycogen concentration prior to performing a TT is a reliable experimental design. Therefore, this study used a TT protocol in order to assess performance as its outcome measure.

2.7 Blood analyses

Blood samples were obtained from participants via an antecubital venous cannula (BD Venflon™, Helsingborg, Sweden). On entering the laboratory participants were fitted with a cannula and a three-way stopcock. During each trial, 10ml samples were obtained on Day 2 at 5 time points; 45-minutes prior to exercise (PRE), immediately prior to the start of exercise (0), at 30 and 60-minute time points during SS and upon completion of the TT (END). Before taking the baseline sample, a 5-minute washout period was adhered to between fitting of the cannula and obtaining the blood sample. This was to allow for any CAT response to cannula insertion to subside. The cannula was flushed after every sample with 5ml saline solution in order to prevent to occurrence of blockage during the trial.

A total of 50ml of blood was withdrawn per trial, thus a total of 150ml was obtained from each participant upon completion of the study. Each 10ml sample was aliquoted into a tube containing ethylenediaminetetraacetic acid (EDTA).

Samples were centrifuged immediately at 3500rpm for 15-minutes at 4$_{o}$C. The cell-free plasma was extracted and separated into microtubes; 400ul of blood plasma was dispensed per microtube. Microtubes were labelled with participant ID, time point and the

trial code (A/B/C). Any remaining plasma was stored in additional microtubes as spare. These samples were then frozen immediately and stored at -70^{o}C for later analysis using a semiautomated analyser.

The cell-free plasma samples were thawed and analysed for determination of metabolite concentrations using enzymatic colorimetric assays using an ILAB 650 Clinical Chemistry Analyser (Instrumentation Laboratory, Bedford, MA). Assays were run for determination of glucose concentration (Glucose Oxidase kit; Instrumentation Laboratories, Cheshire, UK) and NEFA concentration (NEFA kit; Randox Laboratories Ltd., County Antrim, UK).

2.8 Gas analyses

On Day 2 of each experimental trial, measures of $\dot{V}O_2$ and $\dot{V}CO_2$ were taken during the 1-h SS cycling using an automated analyser (JAEGER® Vyntus CPX, CareFusion, GER). Samples were collected throughout SS at 11.5, 26.5, 41.5 and 56.5-minute time points for 3-minutes at a time. Prior to the collection of gases the volume transducer was calibrated with a 3-L calibration syringe (Jaeger) and gas analyser calibration was performed with a 5.07% CO_2, 14.79% O_2, 80.14% N_2 gas mixture (BOC Gasses, Surrey, UK). Collection of both $\dot{V}O_2$ and $\dot{V}CO_2$ (L.min^{-1}) allowed for RER (respiratory quotient [RQ] = $\dot{V}CO_2$ / $\dot{V}O_2$), whole-body rates of fat and CHO oxidation and energy expenditure (EE) over the period of exercise to be determined using Jeukendrup and Wallis' (2005) stoichiometric equations for moderate to high intensity exercise:

$$CHO\ Oxidation = 4.210VCO_2 - 2.962VO_2$$

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

These equations assume a negligible contribution of protein oxidation to metabolism where oxidation rates are in g.min^{-1} and gas exchange in L.min^{-1}.

2.9 Statistical analyses

Data is presented as mean ± standard deviation (SD) and was analysed for statistical significance using the SPSS Statistics software (SPSS Statistics, v24, SPSS Inc., Chicago, IL). Statistical significance was inferred when $P \leq 0.05$, which is indicated symbolically in the results section. All data underwent testing for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965) before any further statistical tests were run using SPSS.

The primary outcome measure of performance (TT time-to-completion) was subject to a one-way repeated-measures analysis of variance (ANOVA). Metabolic factors; RER, CHO oxidation, fat oxidation, NEFA concentration and blood glucose concentration were analysed using two-way repeated-measures ANOVAs. Any data that did not meet the assumption of Mauchly's test of sphericity ($P < 0.05$) was corrected using the Greenhouse-Geisser (< 0.75) or Huynh-Feldt (> 0.75) adjustment (Scheiner and Gurevitch, 2001).

Where a statistically significant effect was identified, the Holm-Bonferroni stepwise correction was used as a post-hoc in order to locate differences. The Holm-Bonferroni correction is a more powerful test than the classical Bonferroni correction (Holm, 1979) and maintains control over the familywise Type 1 error rate (Abdi, 2010). Using this technique, *P*

values were calculated from a simple *t*-test and then multiplied by a correction factor (Holm., 1979). It was appropriate to use the Holm-Bonferroni correction as a post hoc for this data set in order to reduce the risk of a false rejection of any true null hypotheses due to the multiple comparisons.

3.0 Results

3.1 Physiological and Perceptual responses

3.1.1 Day 1 - Depletion

Several variables were monitored during the depletion ride on day 1 of the experimental trials; RPE, HR, time and number of stages completed at each power output. These variables were measured in order to control for any differences in the dependent variables.

RPE was monitored as a perceptual measure of exertion in the last 30-seconds of the 'work' stages of the depletion ride. Table 3 displays the mean RPE throughout the depletion ride in each of the conditions. RPE showed a trend to increase as the intensity of work was reduced, indicating that muscular fatigue was increasing during the depletion until cadence could no longer be maintained, however this trend was not found to be statistically significant ($P > 0.05$).

HR was monitored consistently throughout the depletion ride as a measure of physiological exertion. The mean HR data from each trial condition is displayed in table 3. There was a significant effect of power output on HR ($P < 0.001$). However, there was no significant effect of trial condition on mean HR during the depletion ride ($P = 0.433$).

The mean times to fatigue in the depletion ride are displayed in table 3. There is a small difference in mean time to fatigue between the three trials. The Dep-PLA trial mean depletion ride time was the longest, closely followed by the Rep-CHO trial which was 3.28-minutes shorter. The Dep-CHO trial was the shortest, 3.75-minutes shorter than Dep-PLA and just 0.47-minutes shorter than Rep-CHO. These differences were not statistically

significant (P = 0.847) and therefore this is unlikely to have been a confounding variable on the results of the steady state and time trial on day 2 of the trials.

Table 3. Mean depletion ride time, heart rate, percentage of maximum heart rate and rating of perceived exertion during the work stages of the depletion.

	Depletion ride time (minutes)	HR	%HRmax	RPE
Rep-CHO	131.18±14.49	162±6	86±4	18±1
Dep-CHO	130.71±38.65	163±6	86±4	17±1
Dep-PLA	134.46±28.29	160±6	85±4	18±1

Values are mean ± SD. Abbreviations: HR = heart rate, %HRmax = percentage of maximum heart rate, RPE = rating of perceived exertion.

The number of stages completed by each participant at each 'work' power output was recorded (table 4). There was no significant difference in the number of stages completed at each power output (P = 0.201), nor was there any significant difference between the number of stages completed in each trial condition (P = 0.949).

Table 4. Mean number of stages completed at each workload in the depletion ride.

	Number of stages completed		
	90%Wmax	80%Wmax	70%Wmax
Rep-CHO	12±6	12±5	7±4
Dep-CHO	14±12	8±5	9±4
Dep-PLA	13±9	12±3	6±4

Values are mean ± SD. Abbreviations: %Wmax = percentage of maximum power output.

Table 5 shows the mean total amount of work completed during the depletion ride on day 1 in each of the three different nutritional conditions. There were no significant differences found between trials in the total amount of work completed ($P > 0.05$).

Table 5. Mean total work completed during the depletion exercise on day 1 of the experimental trials in each of the nutritional conditions.

Nutritional Intervention	Total work completed (W)
Rep-CHO	8720±1540
Dep-CHO	8584±2969
Dep-PLA	8857±2450

Values are mean±SD. Abbreviations: W = watts.

3.1.2 Day 2 - Steady state

$\dot{V}O_2$ was monitored throughout the 1-h SS cycling exercise on day 2 of each trial using a metabolic cart. Samples were taken every 15-minutes, at every measurement time-point 3-minutes' worth of gases were monitored. There was no significant effect of time ($P = 0.052$) or nutritional condition ($P = 0.353$) on $\dot{V}O_2$, nor was there any interaction effect between nutritional condition and time ($P = 0.870$). This indicates that the work intensity was well controlled during the SS cycling bout across all trials. However, it must be noted that whilst there was no significant effect of time on $\dot{V}O_2$, the margin by which significance was missed is very small, indicating that oxygen consumption showed a trend to increase over time during the SS bout.

The %$\dot{V}O_2$max data calculated (table 6) reflects the intensity of work during the SS cycling bout and aligns with our prediction that working at 50%Wmax would elicit a workload of between 65-70%$\dot{V}O_2$max. There were no significant differences found in %$\dot{V}O_2$max between trials ($P = 0.235$).

Additionally, there were no significant differences in HR or percentage of maximum heart rate (%HRmax) between conditions ($P = 0.153$).

Table 6. Mean oxygen uptake, percentage of maximal oxygen uptake, heart rate, percentage of maximum heart rate and respiratory exchange ratio during 1-h of steady state cycling on day 2 of the trial.

	V̇O2	%V̇O2max	HR	%HRmax	RER
Rep-CHO	2530±325	65±4	136±9	72±4	0.86±0.06
Dep-PLA	2582±259	66±2	138±8	73±4	0.82±0.02
Dep-PLA	2597±273	67±3	139±9	74±4	0.81±0.04

Values are mean±SD. Abbreviations: % V̇O2max = percentage of maximal oxygen consumption, HR = heart rate, %HRmax = percentage of maximum heart rate, RER = respiratory exchange ratio.

RER was measured at 15-minute time points during 1-h of SS cycling on day 2 of each trial. The data collected is displayed in figure 3. RER was highest in all conditions at the 15-minute time point, and was reduced thereafter at 30, 45 and 60-minutes in Rep-CHO and Dep-CHO, and at 30 and 45-minutes in Dep-PLA ($P < 0.01$ to $P < 0.05$). There was a small increase in RER from 45 to 60-minutes in the Dep-CHO trial only ($P < 0.05$). There was no significant effect of condition on RER during the SS bout of cycling ($P > 0.05$).

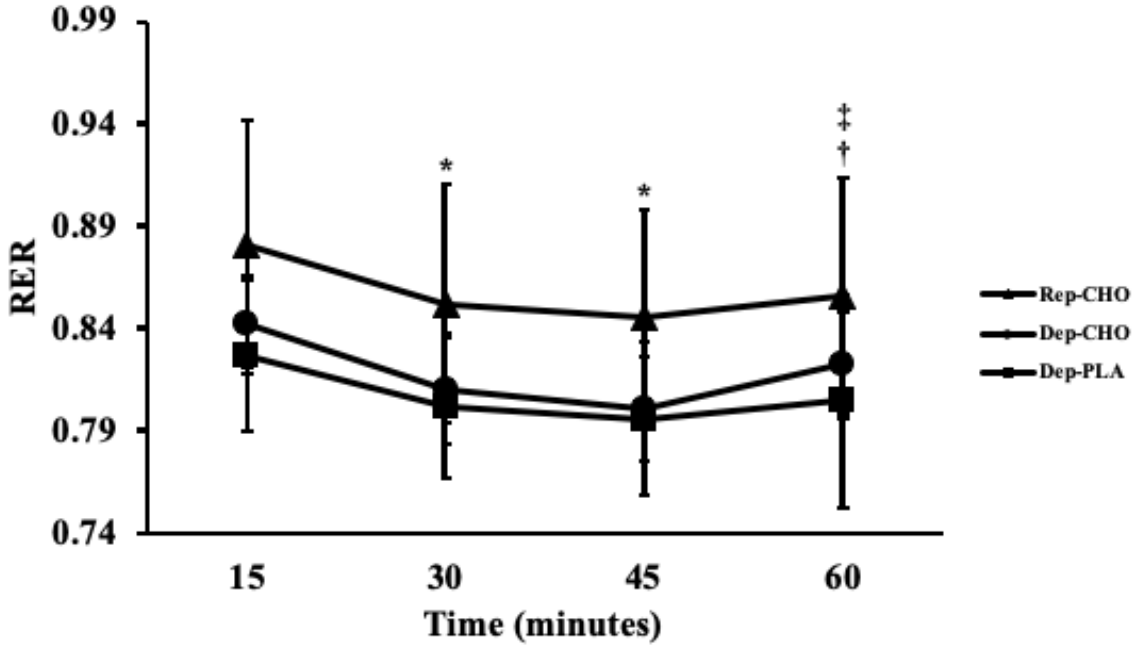

Figure 3. RER (mean±SD, n=8) during 1-h steady state cycling on day 2 of each of the trials. *denotes statistical significance from 15-minutes in all conditions at P < 0.05, †denotes statistical significance from 15-minutes in Rep-CHO and Dep-CHO conditions at P < 0.05, ‡denotes statistical significance from 45-minutes in Dep-CHO condition at P < 0.05.

3.1.3 CHO oxidation

In all three conditions there is a similar pattern of CHO oxidation (figure 4), with oxidation values being consistently higher in the Rep-CHO condition compared to the Dep-CHO and Dep-PLA conditions. However, this elevation did not reach statistical significance. In all three conditions, CHO oxidation was at its peak at the 15-minute time point at which point oxidation was significantly higher than at the 30 and 45-minute time points ($P < 0.05$). In both the Rep-CHO and Dep-CHO conditions CHO oxidation was also significantly greater at 15minutes than at 60-minutes ($P < 0.05$). Additionally, in the Dep-CHO condition CHO

oxidation was significantly increased at 60-minutes compared to at the 45-minute time point ($P < 0.05$).

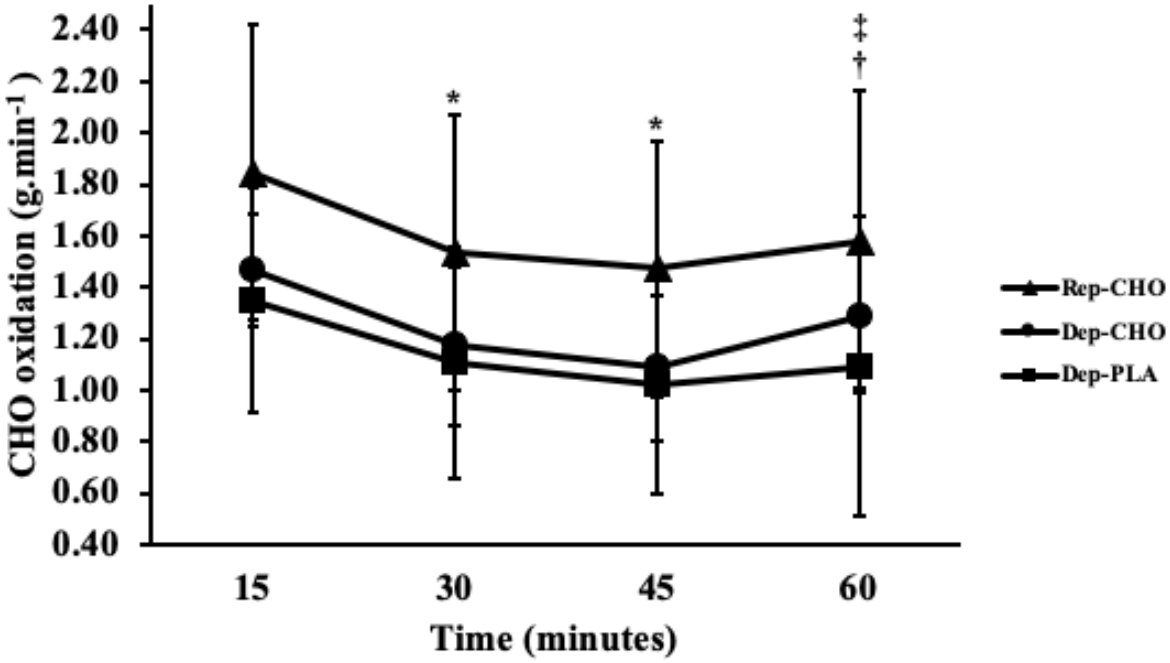

Figure 4. CHO oxidation (mean±SD, n=8) during 1-h steady state cycling on day 2 of the trial in each of the three nutritional intervention conditions. *denotes statistical significance from 15-minutes in all 3 conditions at P < 0.05, †denotes statistical significance from 15-minutes in the Rep-CHO and Dep-CHO conditions at P < 0.05, ‡denotes statistical significance from 45-minutes in the Dep-CHO condition at P < 0.05.

3.1.4 Fat oxidation

Fat oxidation exhibited a mirrored response to CHO oxidation (figure 5), with oxidation values being consistently lowest in the Rep-CHO condition, although never significantly lower than in the Dep-CHO or Dep-PLA conditions. Fat oxidation at the 15-minute time point was significantly greater than at the 30 and 45-minute time points in both the Dep-CHO and Dep-PLA conditions ($P < 0.05$). Conversely, in the Rep-CHO condition oxidation was

significantly lower at the 15-minute time point compared to the 30, 45 and 60-minute time points ($P < 0.05$). Additionally, in the Dep-CHO condition the fat oxidation value at 15minutes was significantly lower than at 60-minutes ($P < 0.05$), and the oxidation value at 45minutes was significantly greater than the 60-minute value ($P < 0.05$).

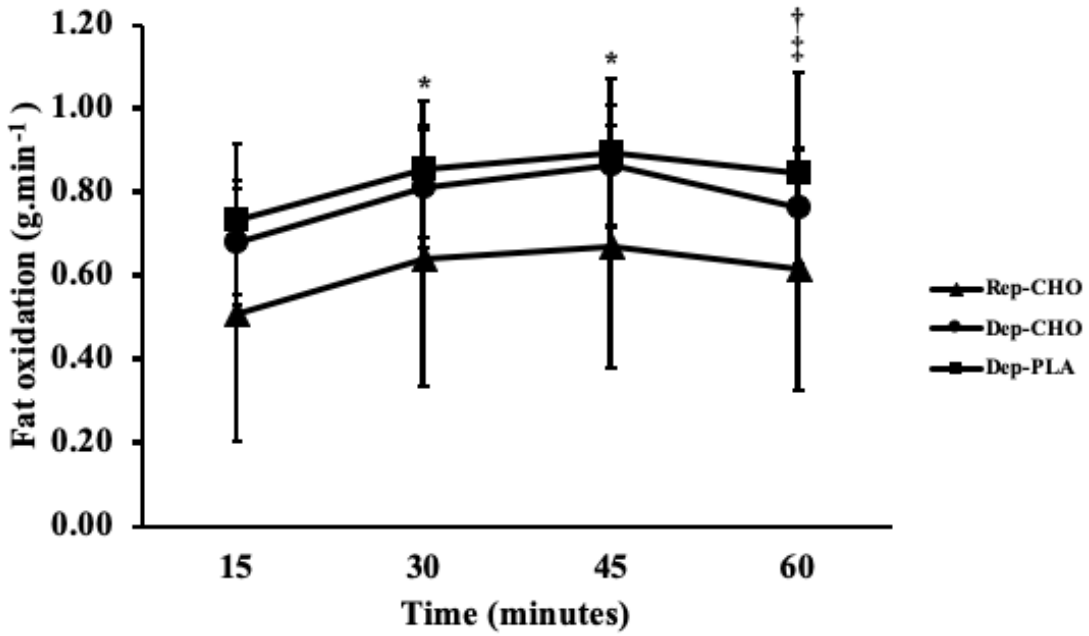

Figure 5. Fat oxidation (mean±SD, n=8) during 1-h steady state cycling on day 2 of the trial in each of the three nutritional intervention conditions. *denotes statistical significance from 15-minutes in all 3 conditions at P < 0.05, †denotes statistical significance from 15-minutes in the Rep-CHO and Dep-CHO conditions at P < 0.05, ‡denotes statistical significance from 45-minutes in the Dep-CHO condition at P < 0.05.

3.2 Blood Metabolic Response

3.2.1 Blood glucose concentration

The blood glucose response varied across all three conditions; data are displayed in figure 6. Plasma glucose concentration remained relatively unchanged in all three conditions from pre-exercise until 30-minutes. Thereafter in the Rep-CHO condition blood glucose concentration showed a trend to increase, however this increase was non-significant. Similarly, in the Dep-CHO condition, blood glucose concentration increased from 30 to 60-minutes and again to the end time point ($P < 0.05$). In the Dep-PLA condition glucose concentration appears to have decreased over time, with the end time point being significantly reduced when compared to all other measurement points ($P < 0.05$). Additionally, both the 60-minute and end time points in the Dep-PLA trial had significantly lower blood glucose concentrations when compared to the concentrations in the Dep-CHO trial at these measurement points ($P < 0.01$).

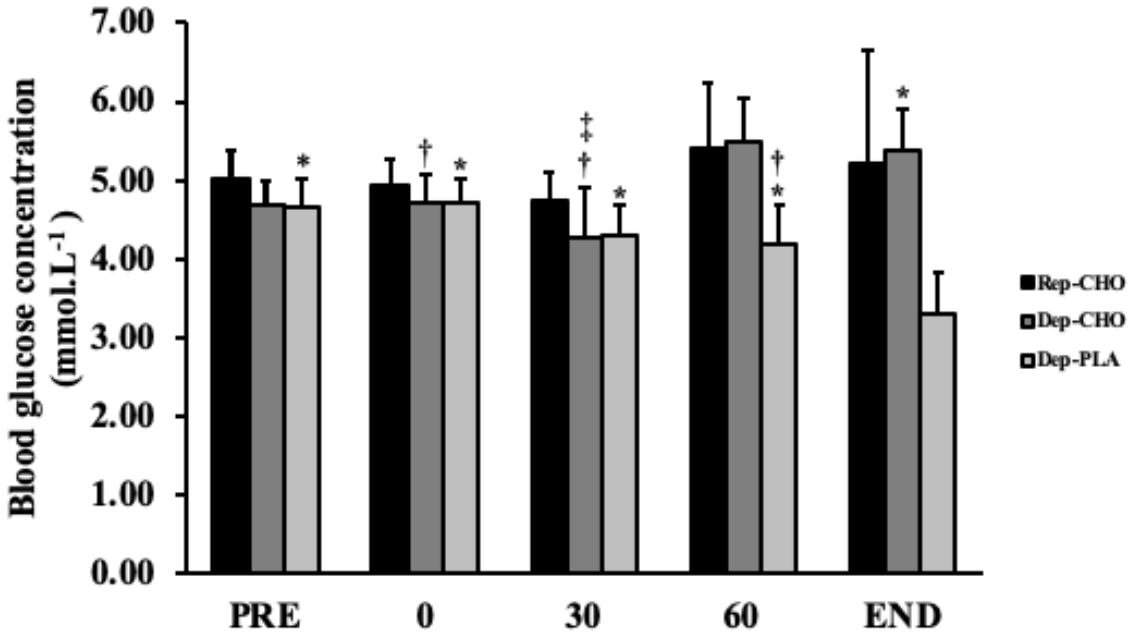

Figure 6. Blood glucose concentration (mean±SD, n=8) measured at multiple time points throughout day 2 of each trial (Rep-CHO, Dep-CHO and Dep-PLA). *denotes statistical significance from end time point in Dep-PLA at P < 0.01, †denotes significance from 60minutes in Dep-CHO at P < 0.05, ‡denotes significance from end time point in Dep-CHO at P < 0.05.

3.2.2. Blood non-esterified fatty acid concentration

Blood NEFA concentration displayed a markedly different response to blood glucose concentration, as shown by data in figure 7. In all three conditions NEFA concentration decreased significantly from the pre time point to 0-minutes ($P < 0.05$). Thereafter in the Rep-CHO trial, NEFA concentration began to rise again following the 0-minute time point. However, this increase was non-significant and NEFA concentration never returned to baseline level (0.97±0.57mmol.L^{-1}). The Dep-CHO condition exhibited a similar NEFA response to the Rep-CHO condition. NEFA concentration increased significantly from 0 to 30-minutes ($P < 0.001$). However, unlike in the Rep-CHO trial, NEFA concentration then fell significantly from 30 to 60-minutes and end point respectively ($P < 0.001$). From the 0-minute time point

onwards, NEFA concentration in the Dep-PLA condition displayed an almost completely converse response when compared to the Rep-CHO condition. NEFA concentration rose significantly from 0-minutes to 30-minutes, 60-minutes and end time point ($P < 0.001$). The NEFA concentration at the end of the Dep-PLA trial (1.38±0.44mmol.L^{-1}) had almost returned to baseline (1.52±0.37mmol.L^{-1}). Additionally, at 60-minutes and the end time point in the Dep-PLA condition, blood NEFA concentration was significantly greater than in the Rep-CHO and the Dep-CHO trials ($P < 0.05$).

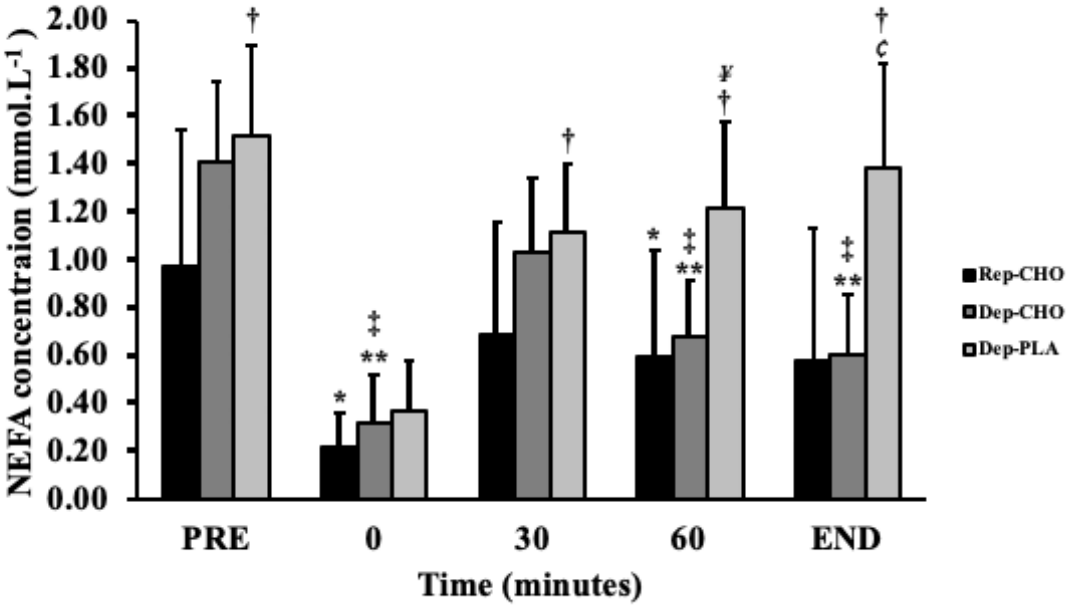

Figure 7. Blood NEFA concentration (mean±SD, n=8) measured at multiple time points throughout day 2 of each trial (Rep-CHO, Dep-CHO and Dep-PLA). *denotes statistical significance from 'pre' time point in Rep-CHO at P < 0.05, **denotes statistical significance from 'pre' time point in Dep-CHO at P < 0.001, †denotes statistical significance from 0-minutes in Dep-PLA at P < 0.001, ‡denotes statistical significance from 30-minutes in Dep-CHO at P < 0.001, ¥ denotes statistical significance from 60-minutes in Rep-CHO and Dep-CHO at P < 0.05, ¢ denotes statistical significance from end time-point in Rep-CHO and Dep-CHO at P < 0.05.

3.3 Time Trial

3.3.1 Time to completion

The mean TT data displayed is only for 7 out of the 8 participants, the data of one of the participants has been omitted from this section due to failure to complete the TT in the Dep-PLA condition. This meant that this participant's data could not be included in the statistical analysis.

Figure 8 depicts the mean time-to-completion of the TT for 7 participants. The mean data indicate that the TT was completed fastest in the Rep-CHO condition (42.15±8.58 minutes), followed by the Dep-CHO condition (44.05±7.68 minutes) and then the Dep-PLA condition (47.74±9.73 minutes). However, there were no significant differences in TT time between the conditions ($P = 0.216$). Despite the direction of these data when presented as group means, the individual responses were highly variable, illustrated in figure 6 where individual TT data has been plotted for 7 participants.

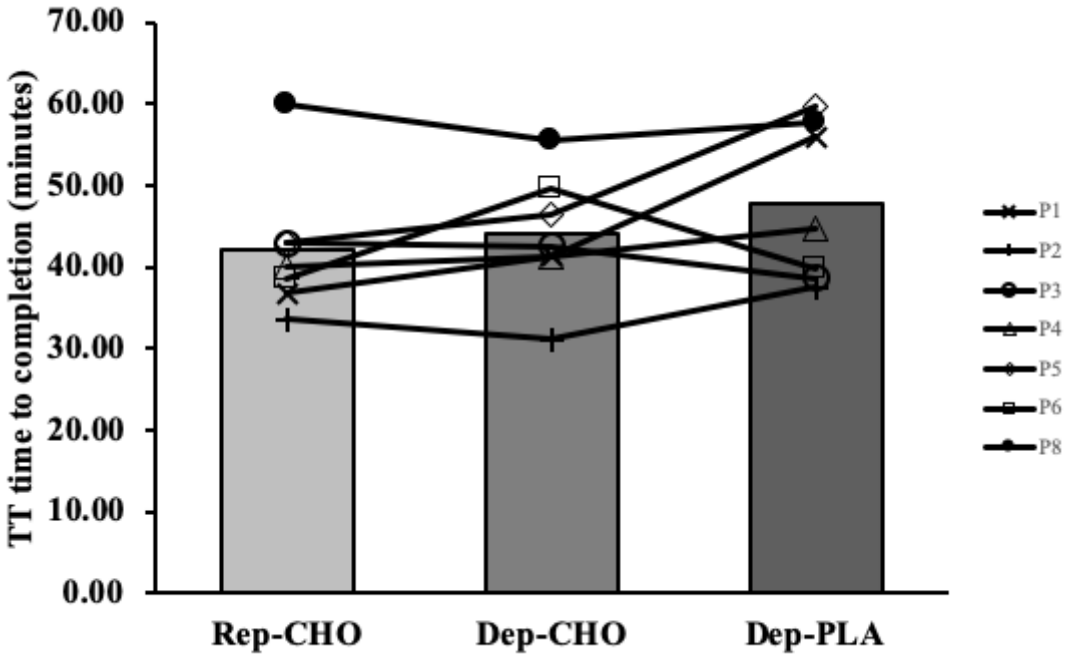

Figure 8. Mean TT time to completion (n=7) in each of the three trials, with individual variability plot. Note that one participant's data has been omitted due to lack of completion of one of the time trials.

3.3.2 Heart rate

Mean HR was monitored throughout the time trial as a measure of physiological exertion (table 7). There was no significant difference in mean HR between the three trial conditions (P = 0.204).

Table 7. Mean heart rate during the TT in each trial.

Nutritional Intervention	HR
Rep-CHO	153±16
Dep-CHO	150±16
Dep-PLA	146±14

Values are mean ± SD. Abbreviations: HR = heart rate, TT = time trial.

3.3.3 Power output

Power output data was gathered from the TT software and used to calculate mean power output and percentage of maximum power output (%Wmax) during the TT (table 8). There were no significant differences in mean power output ($P = 0.191$) or %Wmax between any of the trial conditions ($P = 0.201$).

Table 8. Mean power output and mean %Wmax during the TT in each nutritional condition.

Nutritional Intervention	W	%Wmax
Rep-CHO	219±47	63±11
Dep-CHO	211±50	61±12
Dep-PLA	193±36	56±11

Values are mean ± SD. Abbreviations: W = power output in watts, %Wmax = percentage of maximum power output, TT = time trial.

4.0 Discussion

4.1 Key findings

The purpose of this research was to characterise the metabolic response to delayed CHO feeding during exercise in the glycogen depleted state and to assess the effect on endurance performance. It was hypothesised that markers of lipid metabolism would be upregulated in comparison to the glycogen replete condition and that performance would be improved in the glycogen depleted state when CHO was fed compared to PLA.

There were several key findings of this research study in relation to the experimental hypotheses. Interestingly, NEFA concentration was not increased in the Dep-CHO condition compared to the Rep-CHO condition ($P > 0.05$). A finding that is contrary to the metabolic hypothesis. Additionally, lipid oxidation in the Dep-CHO trial decreased significantly towards the end of the SS exercise bout on day 2 after the ingestion of CHO ($P < 0.05$). Therefore, the hypothesis of an increase in markers of lipid metabolism in the glycogen depleted state compared to the normal glycogen condition cannot be accepted. In relation to the performance hypothesis, it was found that delayed feeding of CHO in the glycogen depleted state did not improve endurance performance in a TT compared to when CHO was not ingested. Thus, this hypothesis also cannot be accepted.

4.2 Metabolic outcomes

A number of metabolic factors were measured in the present study; blood glucose concentration, circulating NEFA concentration, RER, CHO oxidation and fat oxidation.

Based on previous research (Coggan and Coyle, 1989), it was predicted that in a condition where muscle glycogen content was low and CHO was fed after the start of exercise (i.e., a delay), that the metabolic environment would remain unchanged when compared to that produced when exercising in the depleted state without any exogenous CHO provision. The response of blood glucose concentration was varied across all three conditions, whilst it did not differ significantly ($P > 0.05$), there are some interesting observations to be made. Firstly, in all three conditions blood glucose remained stable from before the start of exercise until 30-minutes, at which point CHO was fed in the Rep-CHO and Dep-CHO conditions. Thereafter, blood glucose concentration showed a trend to increase in the Rep-CHO condition however, this increase was not significant ($P > 0.05$). The Dep-PLA condition elicited a mirrored response, with blood glucose concentration decreasing over time, the end measurement point in this trial was significantly lower than all other measurement points within the trial at 3.31±0.53mmol.L^{-1} ($P < 0.05$). The data from these two trials follow the expected pattern, making them ideal comparisons for the Dep-CHO data. In the Dep-CHO condition, the rise in blood glucose concentration was statistically significant from 30 to 60-minutes and from 30-minutes until the end of the TT ($P < 0.05$). Additionally, concentrations at 60-minutes and at the end of the TT in Dep-CHO were significantly greater than in the Dep-PLA condition. This finding implies that feeding CHO in the glycogen depleted state elicits an improved ability to maintain and increase plasma glucose concentration during exercise. This finding is unsurprising as it is well documented in the literature that the ingestion of exogenous CHO during exercise leads to an elevated blood glucose concentration compared to when PLA is ingested (Coyle et al., 1983). Multiple studies have since illustrated that feeding CHO during exercise delays the onset of fatigue and improves performance,

particularly when muscle glycogen content is a limiting factor due to the ability to sustain CHO oxidation (Hargreaves et al., 1984; Coyle, 1992; Stellingwerff et al., 2007).

It is visible from the CHO oxidation data produced in this study that the ingestion of carbohydrate from an exogenous source during exercise leads to increased CHO oxidation. In the Rep-CHO trial, CHO oxidation is consistently higher than in either of the other two conditions. Given that muscle glycogen content in this condition is expected to be higher than the other two conditions from the onset of exercise this finding was expected. CHO oxidation rates in the Dep-PLA and Dep-CHO trials exhibited a similar pattern over time, that is until the 60-minute time point, when in the Dep-CHO trial CHO oxidation then rose significantly ($P < 0.05$). In this trial CHO was supplied after 30-minutes of exercise, therefore the increase in CHO oxidation was not immediate because at the 45-minute time point there is no observable rise in oxidation. This finding correlates well with the blood glucose data, which shows blood glucose concentration to increase significantly from 30 to 60-minutes.

It is interesting that the rise in both blood glucose concentration and CHO oxidation in the Dep-CHO trial is significant, whereas when CHO was fed in the Rep-CHO trial the increases were nonsignificant. It appears likely that pre-exercise muscle glycogen content affects the response to the ingestion of exogenous glucose. Where muscle glycogen is low the response is augmented, whereas when muscle glycogen content is sufficient the response appears to be less exaggerated. It can be speculated that the reason for this differing response is due to the fact that in the Rep-CHO condition, endogenous glucose supply was sufficient to perform the exercise and therefore supplementation with exogenous glucose did not increase CHO oxidation as the rate was already adequate. Whereas, in the Dep-CHO condition where glycogen was depleted, the ingestion of exogenous glucose acted to increase CHO oxidation in order to sustain performance. A possible mechanistic explanation for this outcome is the

effect of low muscle glycogen content during exercise on the utilisation of hepatic glycogen stores. The reliance on liver glycogenolysis as a source of fuel increases in conditions of critically low muscle glycogen stores (Wasserman and Cherrington, 1991), such as the Dep-CHO condition of this study. Additionally, in the Dep-CHO condition exogenous glucose was provided to participants, therefore this supplementation of CHO combined with elevated rates of hepatic glycogenolysis is a possible explanation for the significant rise in oxidation. Whereas, in the Rep-CHO condition where muscle glycogen content is sufficient to sustain exercise, the hepatic contribution to CHO oxidation is minimal. However, it must be noted that the present study did not directly measure muscle or liver glycogen content before or after the depletion exercise and therefore it is not possible to conclude with any certainty that this was the case. Liver glycogen as well as muscular stores may have been significantly depleted on day 1 and it is therefore possible that an alternative mechanism may have been responsible for the increased CHO oxidation observed in the Dep-CHO condition.

The response of circulating NEFA concentration appears to almost mirror the response of blood glucose concentration. In all three experimental conditions NEFA concentration was significantly reduced from pre-trial to the start of exercise ($P < 0.05$). This suppression coincided with the ingestion of a protein gel before the start of the steady state exercise. This is likely to have been responsible for the suppression of circulating NEFA due to a temporary increase in insulin levels, an effect well documented in the literature (Floyd et al., 1966; Fajans et al., 1969). Thereafter in the Dep-PLA condition NEFA levels rose significantly ($P < 0.001$) and returned almost to baseline, just 0.14mmol.L^{-1} below the pre-exercise value. The fat oxidation data observed is consistent with this finding, oxidation in the Dep-PLA trial was consistently higher than that in the Rep-CHO trial, although this difference was not statistically significant ($P > 0.05$). In the Rep-CHO trial, circulating NEFA remained

suppressed after 0-minutes until the end of the time-trial. In accordance with this finding, fat oxidation rates in this trial were consistently lower than in both Dep-PLA and Dep-CHO. Muscle glycogen concentration in this trial was expected to be high, thus lipid oxidation was suppressed in favour of CHO oxidation due to the abundance of endogenous CHO. NEFA concentration in the Dep-CHO trial was not significantly elevated compared to the Rep-CHO trial, showing a trend to decrease significantly after the provision of CHO at 30-minutes ($P < 0.05$), which is the opposite of the response that was predicted.

The fat oxidation data shows a mirrored response to the CHO oxidation data, with oxidation rates in the Rep-CHO trial being consistently lower than in the Dep-CHO or Dep-PLA trials. In both Dep-PLA and Dep-CHO conditions, fat oxidation was lowest at 15minutes and then increased significantly to 30 and 45-minute time points. However, fat oxidation in Dep-CHO peaked at 45-minutes and thereafter fell significantly at the 60-minute measurement point, however remained elevated significantly above the baseline rate at 15-minutes. This response is not surprising, as at the same time-point NEFA levels began to decrease, thus the amount of oxidisable substrate was reduced. This finding aligns with evidence that glycolytic flux regulates lipid oxidation, a possible mechanism for which is reducing fatty acid transport into the mitochondria (Coyle et al., 1997). It is interesting though that fat oxidation did remain significantly elevated at the 45-minute measurement point, as this was after the first dose of CHO had been ingested, this suggests that there might be a threshold dose after which fat oxidation is suppressed. A study by De Bock et al. (2008), similarly found that when CHO was fed in the fasted state, it did not result in an upregulation of fat oxidation but instead lead to reduced rates of glycogen breakdown compared to a fed condition. It is possible that this was the case in the present study, where exogenous CHO

was preferentially oxidised in order to spare any remaining endogenous glycogen whilst maintaining performance.

Whilst fat oxidation in the Dep-CHO trial was not significantly greater than in the Rep-CHO trial there is an obvious trend for it to be consistently elevated. In future research, it would be interesting to see if this difference could reach significance in order to confer a clear metabolic advantage. There is a possibility is that FFAs could be liberated from the IMTG stores, based on the fact that in conditions of low glycogen availability, levels of circulating CATs are increased (Watt and Hargreaves, 2002) this results in an increased activity of HSL and thus increases the liberation of FFAs from the intramuscular depot due to increased rates of lipolysis (Kiens, 2006). This could potentially offset the reduction in NEFA levels and support increased fat oxidation.

This study did not measure insulin concentration; however, it is well-known that increased insulin concentrations result in reduced circulating NEFA and fat oxidation (Floyd et al., 1966; Fajans et al., 1969). Therefore, it is likely that a rise in insulin levels in response to CHO ingestion was responsible for the reduction in both circulating NEFA and fat oxidation observed in the Dep-CHO condition. This study supplemented 75g of CHO during exercise, a dosage that has been evidenced to significantly benefit endurance performance (Newell et al., 2018). However, it must be considered that this dose may have resulted in an insulin response that subsequently inhibited fat oxidation in the glycogen depleted state. It is possible that by reducing this dose of CHO, there might be the potential to maintain plasma glucose concentration and performance whilst also maintaining elevated levels of circulating NEFA and fat oxidation. However, this concept is purely speculative at this point and requires further investigation.

The metabolic findings of the present study do not quite parallel with a similar study conducted by Coggan and Coyle (1989), who demonstrated that by delaying the feeding of CHO it was possible to increase plasma glucose concentration without compromising the beneficial metabolic responses conveyed as a result of exercising in the glycogen depleted state. The Dep-CHO data does show an increasing and better maintained blood glucose concentration compared to Dep-PLA, however NEFA levels and fat oxidation both began to decrease significantly after the ingestion of CHO. Elevated levels of circulating NEFA is suggested to be an important metabolic trigger for adaptation (Philp, Hargreaves and Baar, 2012), therefore implying that feeding CHO after a delay of 30-minutes following the start of exercise in the glycogen depleted state is not a viable method for maintaining the beneficial metabolic responses typically observed when exercise is commenced with low glycogen as in "train low" strategies.

It is important to note that the interpretation of these metabolic outcomes is based on a very limited sample size practicable within the confines of this Masters thesis. Therefore, metabolic outcomes such as NEFA concentration and substrate oxidation which have a large degree of variability may not show clear significant differences where trends appear in the data.

4.3 Performance outcomes

Time-to-completion of a TT was the performance measure used in this study. Participants completed the TT after 1-h of SS cycling, the duration was based on a set amount of work calculated relative to each participant's Wmax. The TT data of one participant was omitted from statistical analysis due to a failure to complete the TT in the Dep-PLA condition.

Therefore, the data generated is based on the results of the other seven participants.

There was no significant difference in RPE, HR, time to fatigue, stages completed or total amount of work completed between trials on the depletion ride ($P > 0.05$). Therefore, it is safe to assume that this was not a confounding factor to performance outcomes on day 2 of the trial. However, as glycogen content of the skeletal muscle was not measured via biopsy in this study, it cannot be confirmed for certain that glycogen depletion to a sufficient level had occurred following the depletion ride. Nevertheless, the protocol used has been shown to be reliable, valid and sensitive (Currell, Jentjens and Jeukendrup, 2006). The SS ride on day 2 of the trial was well controlled. There were no significant differences between conditions in $\dot{V}O_2$ ($P > 0.05$), additionally $\%\dot{V}O_2max$ data for the trials aligned with the estimation that cycling at 50%Wmax would elicit a workload of 65-70%$\dot{V}O_2max$. Furthermore, there were no significant differences in mean HR and %HRmax between trials ($P > 0.05$). This indicates that the intensity of work during the steady state bout was well controlled and could not have confounded the performance data obtained from the subsequent TT or the metabolic data.

There were no significant differences in mean TT time between the three experimental conditions ($P > 0.05$). However, the means were directionally consistent with the expected outcomes; Rep-CHO was the fastest meant time to completion (42.15±8.58-minutes) and Dep-PLA the slowest (47.74±9.73-minutes). Additionally, the individual variability between trials was very high and combined with a small sample size of 7 participants, it is possible that this null result is the product of a type 2 statistical error. This seems likely as it is difficult to conceive any other reason for the lack of a significant difference between performance in the Rep-CHO and Dep-PLA conditions. It is well documented in the literature that feeding CHO during exercise delays fatigue and improves performance, even when pre-exercise glycogen stores are high (Coggan and Coyle, 1987; Wright, Sherman and Dernbach 1991). Additionally,

a recent study by Learsi et al. (2019) demonstrated that performance in a cycling TT was improved with CHO ingestion regardless of whether participants were in a fed or a fasted state. Therefore, it is unusual that in the present study there was no difference in TT performance between a condition of high muscle glycogen with CHO feeding and low muscle glycogen with PLA feeding, when the participants were essentially 'running-on-empty'. In a real-world environment, such as a cycling race, it is reasonable to suggest that a competitor whose pre-race glycogen levels are high and who supplements with CHO regularly throughout would perform better than one who lacked enough endogenous CHO to even complete the race.

In a study by Widrick et al. (1993) a TT protocol was used as a performance measure to evaluate the effect of feeding CHO during exercise commenced with differing resting glycogen concentrations. This study also had a small population of 8, however it was found that when CHO was fed during exercise in the glycogen depleted state subjects were better able to maintain their power output and pace during a TT, therefore improving performance. The authors attributed this to an increased ability to maintain euglycaemia in the final stages of exercise. Interestingly, the blood glucose data from the present study suggests that this should have been the case in the Dep-CHO condition due to elevated concentrations compared with the Dep-PLA condition. However, this did not translate into a significant performance benefit in the current study. Despite the conclusions of Widrick et al. (1993), the lack of an observed benefit to performance of training low is a commonly reported finding in the literature and it is important to note that in the Widrick study intramuscular glycogen concentrations in the 'low glycogen condition' were comparably higher than the values used in more contemporary literature. Additionally, there are multiple studies in existence on the effects of training low on both metabolism and performance that report a disconnect between an observed metabolic benefit and the subsequent lack of any ergogenic performance effects

(Havermann et al., 2006; Van Proeyen et al., 2011). Therefore, the precise reason for a lack of any observable performance benefit is yet to be elucidated.

It has been widely reported in the literature that one of the main consequences of training in the glycogen depleted state is a reduced attainable training intensity during exercise (Burke, 2010). However, the results of this study do not necessarily support this. Whilst there was no performance benefit of the Dep-CHO condition compared to Dep-PLA, there was also no detriment compared to Rep-CHO. It is possible that this finding was due to the training status of the participants in this study, with the mean $\dot{V}O_2max$ being 59.0±4.6ml.kg.min^{-1}. As such, the participants were all highly trained athletes and therefore used to pushing themselves in training and racing scenarios, thus even in the face of low muscle glycogen availability their performance was not adversely affected. However, this finding does also raise the interesting possibility that supplementation with CHO during exercise might have a more significant effect on performance than pre-exercise muscle glycogen concentration. Nonetheless, this finding requires further investigation before any conclusions can be drawn. Additionally, if the same study were to be conducted in an untrained population, it is possible that the results might show a significant difference in performance between conditions. However, the relevance of train low strategies to this population is less than to elite athletes.

One of the eight participants in this study failed to complete a TT in the Dep-PLA condition, as a result this participant's data was not included in the statistical analysis. It is likely that the participant's blood glucose concentration fell to a critical level of hypoglycaemia (2.67±0.53mmol.L^{-1}), at which point lipid oxidation was insufficient to compensate for the profound decline in substrate availability. This participant did however manage to complete the TT in the Dep-CHO condition, illustrating that delayed CHO feeding

in this instance rescued performance in the glycogen depleted state by increasing plasma glucose levels sufficiently to sustain performance in the TT. Contrary to the primary performance outcome of this study, this observation reinforces the issue of reduced attainable work intensity associated with low CHO availability that is widely reported in the literature (Yeo et al., 2008; Hulston et al., 2010). Additionally, this finding furthers the case for a finding of no performance benefit of Dep-CHO versus Dep-PLA conditions being a result of a type 2 statistical error.

Additionally, there were no significant differences between trials in mean HR. However, these means are again directionally consistent and align well with the time-trial duration data. Mean HR was the highest in the Rep-CHO trial (153±16beats.min^{-1}) and lowest in the Dep-PLA trial (146±14beats.min^{-1}). This indicates that the attainable self-selected intensity of work in the TT was greatest when CHO availability was high. There was also no significant difference in power output between conditions during the TT. However, similarly to the mean HR data, these means were directionally consistent with the TT data. A possible explanation for the lack of a significant performance outcome in the present study is the use of a TT as the outcome measure. Whilst this is purported to be the most valid and reliable performance protocol (Jeukendrup et al., 1996), in the context of this study it may not have been the correct protocol to use. Instead, a TTE protocol may have been more suited to the study aims. Despite that fact that TTE protocols are less reliable than a TT, there is an argument to be made in relation to this study that a TTE protocol would be more ecologically valid in the context of cycling performance. Due to the idea that in a cycling race, in order to stay with the group one must be able to maintain a constant work rate for as long as the race takes. Additionally, a study by Hinckson and Hopkins (2005), found TTE protocols to be inherently reliable as an outcome measure. Furthermore, a TTE study may be more likely to

show an improvement in endurance capacity. This is because the increases in CHO oxidation and blood glucose found in the present study appear to be key metabolic factors for improvement in TTE studies.

Despite evidence in the literature to suggest that feeding CHO in the glycogen depleted state might be a viable strategy for improving performance, the findings of this study suggest that this is not the case. There was no performance advantage of the Dep-CHO condition versus Dep-PLA, however there was also no decrement to performance as compared to the Rep-CHO condition.

4.4 Limitations

One of the major limitations of the present study is the small sample size (n = 8). As a result, the data obtained must be interpreted with a certain degree of caution, as the likelihood of a type 2 statistical error having occurred is high. Therefore, the study is at risk of concluding a "false negative" outcome. Additionally, the participants in this study were not all pure cyclists or triathletes and thus the range of training status between participants was relatively high. This may have had an effect on the data collection, as participants of different training statuses may have responded differently to metabolic manipulation.

Additionally, it was difficult to control both the diet and training of participants in the days leading up to experimental visits. Participants were instructed to record a 2-day food, fluid and training diary prior to the first visit and subsequently replicate this as far as possible before each of the remaining visits. However, whilst in theory this should act as a viable control, it is impossible to know for certain whether or not participants adhered to this instruction when unsupervised. Due to the majority of participants being competitive cyclists

and given that the timing of this study coincided with racing season, they may have deviated from the instruction to avoid strenuous exercise for 48-h prior to each experimental visit due to the demands of their usual training programmes. These limitations are largely unavoidable, and precautions were taken to control them as far as possible in the present study.

Following the depletion ride, muscle biopsies to determine the glycogen content of the skeletal muscle were not taken. Instead, glycogen content was assumed to be sufficiently depleted at the end point of the protocol. This assumption was based on research showing this protocol to be a reliable method of reducing intramuscular glycogen stores (Van Hall et al., 2000; Jentjens et al., 2001). However, despite this evidence we cannot be completely certain that glycogen stores were depleted below the threshold level for adaptation (Impey et al., 2018).

There is a possibility that participants became unblinded to the experimental condition that they were in due to the taste of the drinks and jellies that they were provided with. The CHO containing beverages were markedly sweeter than their PLA counterparts and whilst trials were separated by at least a week in order to prevent this, it is possible that participants guessed their trial condition. If a participant had guessed that they were being fed CHO or PLA their performance in the TT would have subsequently been affected due to psychological factors.

Whilst it is acknowledged that there are a number of limitations to the current study, it should be noted that it was conducted as a preliminary proof of concept study in order to show that delayed CHO feeding during exercise in the glycogen depleted state could be a viable training method. The data collected indicate that it could indeed be, however it is clear that much more refining is needed in future studies in order to show this more conclusively.

4.5 Recommendations for future research

The purpose of the present study was to provide data in order to evaluate whether or not delayed ingestion of CHO during exercise in the glycogen depleted state could be a viable strategy of overcoming the limitations to performance of low glycogen concentration whilst maintaining the beneficial metabolic adaptations. This study has shown that ingesting carbohydrate after a delay of 30-minutes into exercise does increase blood glucose concentration and CHO oxidation, however only at the cost of reducing lipid oxidation. This null finding means that further and more refined research is required in order to provide conclusive evidence that consuming CHO and maintaining an elevated lipid utilisation is possible.

Firstly, any future studies investigating the effect of training low on performance and metabolism should aim to recruit a larger study population in order to ensure that the results are not susceptible to a type 2 statistical error. Additionally, a larger sample size would decrease the variability in the results, which would appear particularly important for the performance data.

Secondly, this study did not take muscle biopsies, and therefore did not measure any of the metabolic markers associated with the low glycogen state in the skeletal muscle. This study was unable to investigate the effect of the intervention on many of the molecular pathways involved in the adaptation to low glycogen content, such as the AMPK and PGC-1α pathways. This additional data would enable clarification of mechanisms involved in the findings of the present study, which are currently purely speculative and based on previous research. Additionally, it would be beneficial for any future studies with similar aims to investigate the kinetics of IMTG as well as NEFA, in order to identify whether or not the

liberation and utilisation of IMTG as a fuel source occurs as a result of reduced muscle glycogen availability.

Furthermore, subsequent studies would benefit from measuring insulin concentration in order to give a more comprehensive picture of the metabolic response to the intervention. Future studies should also replicate the protocol with differing doses of CHO during exercise. These alterations to the study would provide evidence as to whether or not there exists an optimal CHO dose for maintaining plasma glucose concentration and performance, whilst also maintaining the beneficial metabolic adaptations of the glycogen depleted state.

Additionally, it would be advisable for a future study to replicate the protocol used in the present study using a TTE measure of exercise capacity rather than a TT. This alteration in method might produce more profound performance effects resulting from the metabolic outcomes.

Any further research undertaken should be conducted in a stringently controlled environment and should measure more in-depth markers of metabolism in order to shed some light on the responsible mechanisms for any subsequent performance effects.

4.6 Conclusions

After consideration of the data collected in this study it is reasonable to conclude that there appears to be an apparent disconnect between metabolic and performance outcomes. The effect of withholding CHO overnight after a depletion protocol on the blood metabolic response is profound, however there is no indication from the performance data of any significant performance effect, which is somewhat surprising. However, the lack of any performance effect is likely to have resulted from the numerous limitations associated with

this research study and outlined in full in the above limitations section. Therefore, two separate conclusions for the 2 research hypotheses can be drawn from the outcomes of the present study:

1. Delayed feeding of CHO in the glycogen depleted state during exercise leads to increased plasma glucose concentration and CHO oxidation, however NEFA concentration and fat oxidation rates appear to be suppressed by the consumption of CHO during exercise, even after a delay of 30-minutes.
2. Delayed CHO feeding in the glycogen depleted state does not confer an ergogenic effect on performance in a TT compared to the ingestion of PLA.

Consequently, both the primary and secondary hypotheses of the present study must be rejected as null. The evidence gathered from this research shows no indication that markers of lipid metabolism were upregulated as a result of the Dep-CHO intervention, nor was there a significant benefit of this intervention to cycling performance in a time-trial.

It is therefore clear that further research is required in order to confirm whether or not there can be a beneficial effect of low CHO availability on outcomes of performance and metabolism.

5.0 Appendix

5.1 Informed consent form

Does carbohydrate feeding during exercise influence endurance performance and whole-body metabolic perturbations when exercise is commenced with low carbohydrate availability?

Location(s):

- School of Sport, Exercise & Rehabilitation Sciences, University of Birmingham

Investigators:

- Tim Podlogar, Bonnie Free, Dr Gareth Wallis

Name:

D.O.B:

I have read the Participant Information Sheet related to this study discussed the investigation with ……. who has explained the procedures to my satisfaction. I am willing to undergo the following procedures or assessments (please initial);

General Health Questionnaire	
Cycling Ergometer Exercise Testing	
Blood withdrawal	
Dietary intervention	

This study will follow ethical and legal practice and all information about you will be handled in confidence. Study data will be stored in locked cabinets in the School of Sport Exercise and Rehabilitation Sciences and/or on University of Birmingham encrypted computers. This data will be held for 10 years and then destroyed in line with University practice. Blood and urine samples collected during the study will be stored in secure freezers only for as long as is needed to complete the required analyses.

I am willing to undergo the investigation but understand that I am free to withdraw at any time up to 7 days after the final test.

Signed……...

Witness……..

Date……..

5.2 General health questionnaire

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. Your answers will be kept strictly confidential.

1	What is your exact date of birth? Day..... Month.....Year……..... So your age is..... Years		
2	How would you describe your ethnicity? …….. *Please refer to ethnicity codes and enter a code*		
3	When did you last see your doctor? In the: Last week..... Last month..... Last six months..... Year..... More than a year.....		
4a	Are you currently taking any prescription medication?	YES	NO
4b	Are you currently taking any non-prescription medication or nutritional supplements? If yes – which: _____	YES	NO
5	Has your doctor ever advised you not to perform vigorous exercise?	YES	NO
6	Has your doctor ever said you have “heart trouble”?	YES	NO
7	Has your doctor ever said you have high blood pressure?	YES	NO
8	Have you ever taken medication for blood pressure or your heart?	YES	NO
9	Do you feel pain in your chest when you undertake physical activity?	YES	NO
10	In the last month have you had pains in your chest when not doing any physical activity?	YES	NO
11	Has your doctor (or anyone else) said that you have raised blood cholesterol?	YES	NO
12	Have you had a cold or feverish illness in the last month?	YES	NO
13	Do you ever lose balance because of dizziness, or do you ever lose consciousness?	YES	NO
14	a) Do you suffer from back pain b) If so, does it ever prevent you from exercising?	YES YES	NO NO
15	Do you suffer from asthma?	YES	NO
16	Do you have any joint or bone problems that may be made worse by exercise?	YES	NO
17	Has your doctor ever said you have diabetes?	YES	NO
18	Have you ever had viral hepatitis?	YES	NO
19	Do you have any bleeding disorders?	YES	NO
20	Have you previously donated blood (last 12 weeks)?	YES	NO
21	Do you have any known food allergies or intolerances?	YES	NO
22	Do you know of any reason, not mentioned above, why you should not exercise?	YES	NO
23	Do you perform regular physical activity?	YES	NO
24	Are you currently participating in another clinical study?	YES	NO
25	Do you have a history of substance abuse or engagement in uncommon eating practices (e.g., sustained periods of fasting)?	YES	NO
26	Do you have problems with someone taking blood from you?	YES	NO
27	Are you a smoker?	YES	NO

5.3 Borg's RPE Scale

6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

5.4 Food and fluid consumption log

Please fill in this food and fluid consumption log on the day of the familiarisation visit and on the day before it. Please fill in as much information as possible, so that you will be able to replicate the same diet before Experimental visits. You can help yourself with a kitchen scale if you wish so.

Record all the foods and beverages. Please note, 24-h prior to the laboratory visit you are not allowed to drink any beverages containing either caffeine (e.g. such as coffee or tea) or alcohol.

DAY 1:

Time	**Meal**	**What?**	**How much?**	**Preparation**

DAY 2:

Meal	Time	Food	How much	Preparation

Reference List

Abdi, H. (2010). 'Holm's Sequential Bonferroni Procedure'. *Encyclopedia of Research Design,* 1(8), pp.1-8.

Akerstrom, T.C.A., Birk, J.B., Klein, D.K., Erikstrup, C., Plomgaard, P., Pedersen, B.K. and Wojtaszewski, J.F.P. (2006). 'Oral glucose ingestion attenuates exercise-induced activation of 5'-AMP-activated protein kinase in human skeletal muscle'. *Biochemical and Biophysical Research Communications,* 342(3), pp.949-955.

Ataide-Silva, T., Ghiarone, T., Bertuzzi, R., Stathis, C.G., Leandro, C.G. and Lima-Silva, A.E. (2016). 'CHO Mouth Rinse Ameliorates Neuromuscular Response with Lower Endogenous CHO Stores'. *Medicine and Science in Sports and Exercise*, 48(9), pp.18101820.

Bartlett, J.D., Hawley, J.A. and Morton, J.P. (2015). 'Carbohydrate availability and exercise training adaptation: Too much of a good thing?' *European Journal of Sport Science,* 15(1), pp.3-12.

Beelen, M., Burke, L.M., Gibala, M.J. and van Loon, L.J. (2010). 'Nutritional strategies to promote postexercise recovery'. *International Journal of Sport Nutrition and Exercise Metabolism,* 20(6), pp.515-532.

Bergstrom, J., Hermansen, L., Hultman, E. and Saltin, B. (1967). 'Diet, muscle glycogen and physical performance'. *Acta Physiologica Scandinavica,* 71(2-3), pp.140-150.

Bergstrom, J. and Hultman, L. (1966). 'Muscle glycogen synthesis after exercise: An enhancing factor localised to the muscle cells in man'. *Nature,* 210(5033*),* pp.309-310.

Bergstrom, J. and Hultman, E. (1967). 'A study of the glycogen metabolism during exercise in man'. *Scandinavian Journal of Clinical Laboratory Investigations,* 19(3), pp.218-228.

Bernard, C. (1855). '*Leçons de physiologie expérimentale appliquée à la médecine: faites au Collège de France'*. JB Baillière et fils.

Bjorkman, O., Sahlin, K., Hagenfeldt, L. and Wahren, J. (1984). 'Influence of glucose and fructose ingestion on the capacity for long-term exercise in well-trained men'. *Clinical Physiology*, 4(6), pp.483-494

Borg, G.A. (1982). 'Psychophysical bases of perceived exertion'. *Medicine & Science in Sports & Exercise,* 14(5), pp.377-381.

Bosch, A.N., Dennis, S.C. and Noakes, T.D. (1994). 'Influence of carbohydrate ingestion on fuel substrate turnover and oxidation during prolonged exercise'. *Journal of Applied Physiology,* 76(6), pp.2364-2372.

Brown, A.M. and Ransom, B.R. (2007). 'Astrocyte glycogen and brain energy metabolism'. *Glia*, 55(12), pp.1263-1271.

Burke, L.M. (2010). 'Fuelling strategies to optimise performance: training high or training low?' *Scandinavian Journal of Medicine and Science in Sports,* 20(Suppl.2), pp.48-58.

Burke, L.M., Cox, G.R., Cummings, N.K. and Desbrow, B. (2001). 'Guidelines for Daily Carbohydrate Intake'. *Sports Medicine*, 31(4), pp.267-299.

Burke, L.M., Hawley, J.A., Wong, S.H.S. and Jeukendrup, A.E. (2011). 'Carbohydrates for training and competition'. *Journal of Sport Sciences,* 29(sup1), pp.S17-S27.

Burke, L.M, Ross, M.L., Garvican-Lewis, L.A., Welvaert, M., Heikura, I.A., Forbes, S.G., Mirtschin, J.G., Cato, L.E., Strobel, N., Sharma, A.P. and Hawley, J.A. (2010). 'Low carbohydrate, high fat diet impairs exercise economy and negates the performance benefit from intensified training in elite race walkers'. *The Journal of Physiology,* 595(9), pp.2785-2807.

Burke, L.M., van Loon, L.J. and Hawley, J.A. (2016). 'Postexercise muscle glycogen resynthesis in humans'. *Journal of Applied Physiology,* 122(5), pp.1055-1067.

Cataldo, A.M. and Broadwell, R.D. (1986). 'Cytochemical identification of cerebral glycogen and glucose-6-phosphatase activity under normal and experimental conditions: I. Neurons and glia'. *Journal of Electron Microscopy Technique*, 3(4), pp.413-437.

Christensen, E.H. and Hansen, O. (1939). 'III. Arbeitsfahigkeit und Errichtung'. *Scandinavian Journal of Physiology*, 81(1), pp.160-171.

Close, G.L., Hamilton, L., Philp, A., Burke, L.M. and Morton, J.P. (2016). 'New strategies in sport nutrition to increase exercise performance'. *Free Radical Biology and Medicine,* 98, pp.144-158.

Cluberton, L.J., McGee, S.L., Murphy, R.M. and Hargreaves, M. (2005). 'Effect of carbohydrate ingestion on exercise-induced alterations in metabolic gene expression'. *Journal of Applied Physiology*, 99(4), pp.1359-1363.

Cochran, A.J., Myslik, F., MacInnis, M.J., Percival, M.E., Bishop, D., Tarnopolsky, M.A. and Gibala, M.J. (2015). 'Manipulating carbohydrate availability between twice-daily sessions of high-intensity interval training over 2 weeks improves time-trial performance'. *International Journal of Sport Nutrition and Exercise Metabolism,* 25(5), pp.463-470

Coggan, A.R. and Coyle, E.F. (1987). 'Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion'. *Journal of Applied Physiology*, 63(6), pp.2388-2395.

Coggan, A.R. and Coyle, E.F. (1988). 'Effect of carbohydrate feedings during high-intensity exercise'. *Journal of Applied Physiology*, 65(4), pp.1703-1709.

Coggan, A.R. and Coyle, E.F. (1989). ‘Metabolism and performance following carbohydrate ingestion late in exercise’. *Medicine and Science in Sport and Exercise,* 21(1), pp.59-65.

Cori, C.F. (1926). ‘The fate of sugar in the animal body III. The rate of glycogen formation in the liver of normal and insulinized rats during the absorption of glucose, fructose, and galactose’. *Journal of Biological Chemistry*, 70(2), pp.577-585.

Coyle, E.F. (1992). ‘Carbohydrate Supplementation during Exercise’. *The Journal of Nutrition,* 122(3), pp.788-795.

Coyle, E.F., Coggan, A.R., Hemmert, M.K. and Ivy, J.L. (1986). ‘Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate’. *Journal of Applied Physiology,* 61(1), pp.165-172.

Coyle, E.F., Hagberg, J.M., Hurley, B.F., Martin, W.H., Ehsani, A.A. and Holloszy, J.O. (1983). ‘Carbohydrate feeding during prolonged strenuous exercise can delay fatigue’. *Journal of Applied Physiology*, 55(1), pp.230-235.

Coyle, E.F., Jeukendrup, A.E., Wagenmakers, A.J. and Saris, W.H. (1997). ‘Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise’. *American Journal of Physiology-Endocrinology And Metabolism*, 273(2), pp.E268-E275.

Currell, K., Jentjens, R.L.P.G. and Jeukendrup, A.E. (2006). 'Reliability of a cycling time trial in a glycogen-depleted state'. *European Journal of Applied Physiology*, 98(6), pp.583-589.

Currell, K. and Jeukendrup, A.E. (2008). 'Validity, reliability and sensitivity of measures of sporting performance'. *Sports Medicine*, 38(4), pp.297-316.

De Bock, K., Derave, W., Eijnde, B.O., Hesselink, M.K., Koninckx, E., Rose, A.J., Schrauwen, P., Bonen, A., Richter, E.A. and Hespel, P. (2008). 'Effect of training in the fasted state on metabolic response during exercise with carbohydrate intake'. *Journal of Applied Physiology*, 104(4), pp.1045-1055.

Décombaz, J., Jentjens, R., Ith, M., Scheurer, E., Buhler, T., Jeukendrup, A. and Boesch, C. (2011). 'Fructose and Galactose Enhance Postexercise Human Liver Glycogen Synthesis'. *Medicine & Science in Sports & Exercise*, 43(10), pp.1964-1971.

Erickson, M.A., Schwarzkopf, R.J. and McKenzie, R.D. (1987). 'Effects of caffeine, fructose, and glucose ingestion on muscle glycogen utilization during exercise'. *Medicine and Science in Sports and Exercise*, 19(6), pp.579-583.

Fajans, S.S., Floyd Jr, J.C., Knopf, R.F. and Conn, J.W. (1969). 'Effect of amino acids and proteins on insulin secretion in man'. In *Schering Symposium on Endocrinology, Berlin, May 26 to 27, 1967* (pp. 231-238).

Fielding, R.A., Costill, D.L., Fink, W.J., King, D.S., Hargreaves, M. and Kovaleski, J.E. (1985). 'Effect of carbohydrate feeding frequencies and dosage on muscle glycogen use during exercise'. *Medicine and Science in Sports and Exercise*, 17(4), pp.472-476.

Floyd, J.C., Fajans, S.S., Conn, J.W., Knopf, R.F. and Rull, J. (1966). Insulin secretion in response to protein ingestion. *The Journal of Clinical Investigation*, 45(9), pp.1479-1486.

Flynn, M.G., Costill, D.L., Hawley, J.A., Fink, W.J., Neufer, P.D., Fielding, R.A. and Sleeper, M.D. (1987). 'Influence of selected carbohydrate drinks on cycling performance and glycogen use'. *Medicine and Science in Sports and Exercise*, 19(1), pp.37-40.

Fuchs, C.J., Gonzalez, J.T., Beelan, M., Cermak, N.M., Smith, F.E., Thelwall, P.E., Taylor, R. Trenell, M.I., Stevenson, E.J. and van Loon, L.J.C. (2016). 'Sucrose ingestion after exhaustive exercise accelerates liver, but not muscle glycogen repletion compared with glucose ingestion in trained athletes'. *Journal of Applied Physiology*, 120(11), pp.1328-1334.

Goldstein, E.R., Ziegenfuss, T., Kalman, D., Kreider, R., Campbell, B., Wilborn, C., Taylor, L., Willoughby, D., Stout, J., Graves, B.S. and Wildman, R. (2010). 'International Society of Sports Nutrition Position Stand: Caffeine and Performance'. *Journal of the International Society of Sports Nutrition*, 7(1), p.5.

Hansen, A.K., Fischer, C.P., Plomgaard, P., Andersen, J.L., Saltin, B. and Pedersen, B.K. (2005). 'Skeletal muscle adaptation: Training twice every second day vs. training once daily'. *Journal of Applied Physiology*, 98(1), pp.93-99.

Hargreaves, M. and Briggs, C.A. (1988). 'Effect of carbohydrate ingestion on exercise metabolism'. *Journal of Applied Physiology*, 65(4), pp.1553-1555.

Hargreaves, M., Costill, D.L., Coggan, A., Fink, W.J. and Nishibata, I. (1984). 'Effect of carbohydrate feedings on muscle glycogen utilization and exercise performance'. *Medicine and Science in Sports and Exercise*, 16(3), pp.219-22.

Havermann, L., West, S.J., Goedecke, J.H., Macdonald, I.A., St Clair Gibson, A., Noakes, T.D., Lambert, E.V. (2006). 'Fat adaptation followed by carbohydrate loading compromises high-intensity sprint performance'. *Journal of Applied Physiology*, 100(1), pp.194–202.

Hawley, J.A. & Morton, J.P. (2014). 'Ramping up the signal: promoting endurance training adaptation in skeletal muscle by nutritional manipulation'. *Clinical and Experimental Pharmacology & Physiology*, 41(8), pp.608-613.

Hinckson, E.A. and Hopkins, W.G. (2005). 'Reliability of time to exhaustion analyzed with critical-power and log-log modeling'. *Medicine and Science in Sports and Exercise*, 37(4), pp.696-701.

Holm, S. (1979). ‘A simple sequentially rejective multiple test procedure’. *Scandinavian Journal of Statistics*, 6(2), pp.65-70.

Howarth, K.R., Moreau, N.A., Phillips, S.M. and Gibala, M.J. (2009). ‘Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans’. *Journal of Applied Physiology*, 106(4), pp.13941402.

Hulston, C., Venables, M., Mann, C., Martin, C., Philip, A., Barr, K. and Jeukendrup, A. (2010). ‘Training with low muscle glycogen enhances fat metabolism in well-trained cyclists’. *Medicine and Science in Sport and Exercise*, 42(11), pp. 2046-2055.

Hultman, E. and Nilsson, L.H. (1971). ‘Liver glycogen in man. Effect of different diets and muscular exercise’. In *Muscle Metabolism During Exercise* (pp. 143-151). Springer, Boston, MA.

Ishii, S., IIzuka, K., Miller, B.C. and Uyeda, K. (2004). ‘Carbohydrate response element binding protein directly promotes lipogenic enzyme gene transcription’. *Proceedings of the National Academy of Sciences*, 101(44), pp.15597-15602.

Impey, S.G., Hearris, M.A., Hammond, K.M., Bartlett, J.D., Louis, J., Close, G.L. and Morton, J.P. (2018). ‘Fuel for the work required: a theoretical framework for carbohydrate periodization and the glycogen threshold hypothesis’. *Sports Medicine*, 48(5), pp.1031-1048.

Ivy, J.L., Costill, D.L., Fink, W.J. and Lower, R.W. (1979). 'Influence of caffeine and carbohydrate feedings on endurance performance'. *Pulse*, 1620(16.18), p.1693.

Ivy, J.L., Miller, W., Dover, V., Goodyear, L.G., Sherman, W.M., Farrell, S. and Williams, H. (1983). 'Endurance improved by ingestion of a glucose polymer supplement'. *Medicine and Science in Sports and Exercise,* 15(6), pp.466-471.

Jentjens, R.L., Van Loon, L.J., Mann, C.H., Wagenmakers, A.J. and Jeukendrup, A.E. (2001). 'Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis'. *Journal of Applied Physiology*, 91(2), pp.839846.

Jeukendrup, A.E. (2017). 'Periodised Nutrition for Athletes'. *Sports Medicine,* 47(Suppl.1), pp.51-63.

Jeukendrup, A.E., Rollo, I. and Carter, J.M. (2013). 'Carbohydrate mouth rinse: performance effects and mechanisms'. *Sports Science Exchange,* 26(118), pp.1-8.

Jeukendrup, A.E., Saris, W.H.M., Brouns, F. and Kester, A.D.M. (1996). 'A new validated endurance performance test'. *Medicine and Science in Sports and Exercise,* 28(2), pp.266-270.

Jeukendrup, A.E., Wagenmakers, A.J., Stegen, J.H., Gijsen, A.P., Brouns, F. and Saris, W.H. (1999). 'Carbohydrate ingestion can completely suppress endogenous glucose production during exercise'. *American Journal of Physiology-Endocrinology And Metabolism*, 276(4), pp.E672-E683.

Jeukendrup, A.E and Wallis, G.A. (2005). 'Measurement of substrate oxidation during exercise by means of gas exchange measurements'. *International Journal of Sports Medicine,* 26(S1), pp.S28-S37.

Kiens, B., 2006. 'Skeletal muscle lipid metabolism in exercise and insulin resistance'. *Physiological Reviews*, 86(1), pp.205-243.

Krogh, A. and Lindhard, J. (1920). 'The relative value of fat and carbohydrate as sources of muscular energy'. *Biochemical Journal,* 14(3-4), pp.290.

Kuipers, H., Saris, W.H., Brouns, F., Keizer, H.A., ten Bosch, C. (1989). 'Glycogen synthesis during exercise and rest with carbohydrate feeding in males and females'. *International Journal of Sports Medicine,* 10*(*Suppl.1), pp.S63-67.

Lane, S.C., Areta, J.L., Bird, S.R., Coffey, V., Burke, L., Desbrow, B., Karagounis, L.G. and Hawley, J.A. (2013). 'Caffeine ingestion and cycling power output in a low or normal muscle glycogen state'. *Medicine and Science in Sports and Exercise,* 45(8), pp.1577-1584.

Lane, S.C., Camera, D.M., Gray Lassiter, D., Areta, J.L., Bird, S.R., Yeo, W.K., Jeacocke, N.A., Krook, A., Zierath, J.R., Burke, L.M. and Hawley, J.A. (2015). 'Effects of sleeping with reduced carbohydrate availability on acute training responses'. *Journal of Applied Physiology,* 119(6), pp.643-655.

Langenfeld, M.E., Seifert, J.G., Rudge, S.R. and Bucher, R.J. (1994). 'Effect of carbohydrate ingestion on performance of non-fasted cyclists during a simulated 80-mile time trial'. *The Journal of Sports Medicine and Physical Fitness*, 34(3), pp.263-270.

Learsi, S.K., Ghiarone, T., Silva-Cavalcante, M.D., Andrade-Souza, V.A., Ataide-Silva, T., Bertuzzi, R., de Araujo, G.G., McConell, G. and Lima-Silva, A.E. (2019). 'Cycling time trial performance is improved by carbohydrate ingestion during exercise regardless of a fed or fasted state'. *Scandinavian Journal of Medicine & Science in Sports*, 29(5), pp.651-662.

Levine, S.A., Gordon, B. and Derick, C.L. (1924). 'Some changes in the chemical constituents of the blood following a marathon race: with special reference to the development of hypoglycemia'. *Journal of the American Medical Association*, 82(22), pp.1778-1779.

MacIntosh, B.R. and Wright, B.M. (1995). 'Caffeine ingestion and performance of a 1,500-metre swim'. *Canadian Journal of Applied Physiology*, 20(2), pp.168-177.

Marquet, L.A, Brisswalter, J., Louis, J., Tiollier, E., Burke, L.M., Hawley, J.A. and Hausswirth, C. (2016a). 'Enhanced Endurance Performance by Periodisation of Carbohydrate Intake: "Sleep Low" Strategy'. *Medicine and Science in Sports and Exercise*, 48(4), pp.663-672.

Marquet, L.A., Hausswirth, C., Molle, O., Hawley, J.A., Burke, L.M., Tiollier, E. and Brisswalter, J. (2016b). 'Periodisation of Carbohydrate Intake: Short-Term Effect on Performance'. *Nutrients,* 8(12), pp.755.

Meyer, C. and Gerich, J.E. (2000). 'Role of the human kidney in glucose homeostasis'. *Current Opinion in Endocrinology, Diabetes and Obesity*, 7(1), pp.19-24.

Mitchell, J.B., Costill, D.L., Houmard, J.A., Fink, W.J., Pascoe, D.D. and Pearson, D.R. (1989). 'Influence of carbohydrate dosage on exercise performance and glycogen metabolism'. *Journal of Applied Physiology*, 67(5), pp.1843-1849.

Morton, J.P., Croft, L., Bartlett, J.D., MacLaren, D.P., Reilly, T., Evans, L., McArdle, A. and Drust, B. (2009). 'Reduced carbohydrate availability does not modulate traininginduced heat shock protein adaptations but does upregulate oxidative enzyme activity in human skeletal muscle'. *Journal of Applied Physiology,* 106(5), pp.1513-1521.

Newell, M., Wallis, G.A. and Galloway, S.D. (2014). 'Impact of carbohydrate nutrition on exercise metabolism and performance'. *Agro Food Industry Hi Tech*, 25(2), pp.32-38.

Newell, M.L., Wallis, G.A., Hunter, A.M., Tipton, K.D. and Galloway, S.D. (2018). 'Metabolic responses to carbohydrate ingestion during exercise: Associations between carbohydrate dose and endurance performance'. *Nutrients*, 10(1), p.37.

Philp, A., Hargreaves, M. and Baar, K. (2012). 'More than a store: regulatory roles for glycogen in skeletal muscle adaptation to exercise'. *Applied Journal of Physiology: Endocrinology and Metabolism,* 302(11), pp.E1343-E1351.

Philp, A., MacKenzie, M.G., Belew, M.Y., Towler, M.C., Corstorphine, A., Papalamprou, A., Hardie, D.G. and Baar, K. (2013). 'Glycogen content regulates peroxisome proliferator activated receptor-y (PPAR-y) activity in rat skeletal muscle'. *PLOS one,* 8(10), pp.e77200.

Pilegaard, H., Osada, T., Andersen, L.T., Helge, J.W., Saltin, B. and Neufer, P.D. (2005). 'Substrate availability and transcriptional regulation of metabolic genes in human skeletal muscle during recovery from exercise'. *Metabolism: Clinical and Experimental,* 54(8), pp.1048-1055.

Psilander, N., Frank, P., Flockhart, M. and Sahlin, K. (2013). 'Exercise with low glycogen increases PGC-1a gene expression in human skeletal muscle'. *European Journal of Applied Physiology,* 113(4), pp.951-963.

Rigden, D.J., Jellyman, A.E., Frayn, K.N. and Coppack, S.W. (1990). 'Human adipose tissue glycogen levels and responses to carbohydrate feeding'. *European Journal of Clinical Nutrition*, 44(9), pp.689-692.

Scheiner, S. and Gurevitch, J. (2001). *Design and Analysis of Ecological Experiments*. New York: Oxford University Press, pp.143-144.

Shapiro, S.S. and Wilk, M.B. (1965). ‘An analysis of variance test for normality (complete samples)’. *Biometrika,* 52(3/4), pp.591-611.

Smith, J.W., Zachwieja, J.J., Péronnet, F., Passe, D.H., Massicotte, D., Lavoie, C. and Pascoe, D.D. (2010). ‘Fuel selection and cycling endurance performance with ingestion of [13C] glucose: evidence for a carbohydrate dose response’. *Journal of Applied Physiology*, 108(6), pp.1520-1529.

Stellingwerff, T., Boon, H., Gijsen, A.P., Stegen, J.H., Kuipers, H. and van Loon, L.J. (2007). ‘Carbohydrate supplementation during prolonged cycling exercise spares muscle glycogen but does not affect intramyocellular lipid use’. *European Journal of Physiology*, 454(4), pp.635-647.

Tabata, I., Atomi, Y. and Miyashita, M. (1984). ‘Blood glucose concentration dependent ACTH and cortisol responses to prolonged exercise’. *Clinical Physiology*, 4(4), pp.299-307.

Tsintzas, K., Liu, R., Williams, C., Campbell, I. and Gaitanos, G. (1993). ‘The effect of carbohydrate ingestion on performance during a 30-km race’. *International Journal of Sport Nutrition*, 3(2), pp.127-139.

Tsintzas, K. and Williams, C. (1998). 'Human muscle glycogen metabolism during exercise. Effect of carbohydrate supplementation'. *Sports Medicine (Auckland, N.Z.),* 25(1), pp.7-23.

Tsintzas, O.K., Williams, C., Boobis, L. and Greenhaff, P. (1995). 'Carbohydrate ingestion and glycogen utilization in different muscle fibre types in man'. *The Journal of Physiology*, 489(1), pp.243-250.

Tsintzas, O.K., Williams, C., Boobis, L. and Greenhaff, P. (1996). 'Carbohydrate ingestion and single muscle fiber glycogen metabolism during prolonged running in men'. *Journal of Applied Physiology,* 81(2), pp.801-809.

Van der Vies, J. (1954). 'Two methods for the determination of glycogen in liver'. *Biochemical Journal*, 57(3), p.410.

Van Hall, G., Shirreffs, S.M., Calbet, J.A. (2000). 'Muscle glycogen resynthesis during recovery from cycle exercise: no effect of additional protein ingestion'. *Journal of Applied Physiology,* 88*(*5), pp.1631-1636.

van Loon, L.J., Jeukendrup, A.E., Saris, W.H. and Wagenmakers, A.J. (1999). 'Effect of training status on fuel selection during submaximal exercise with glucose ingestion'. *Journal of Applied Physiology*, 87(4), pp.1413-1420.

van Loon, L.J., Saris, W.H., Kruijshoop, M. and Wagenmakers, A.J. (2000). 'Maximising postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures'. *American Journal of Clinical Nutrition*, 72(1), pp.106-111.

Van Proeyen, K., Szlufcik, K., Nielens, H., Ramaekers, M. and Hespel, P. (2011). 'Beneficial metabolic adaptations due to endurance exercise training in the fasted state'. *Journal of Applied Physiology (Bethesda, Md.:1985)*, 110, pp.236-245.

Wallis, G.A., Hulston, C.J., Mann, C.H., Roper, H.P., Tipton, K.D. and Jeukendrup, A.E. (2008). 'Postexercise muscle glycogen synthesis with combined glucose and fructose ingestion'. *Medicine and Science in Sports and Exercise*, 40(10), pp.1789-1794.

Wasserman, D.H. and Cherrington, A.D. (1991). 'Hepatic fuel metabolism during muscular work: role and regulation'. *American Journal of Physiology-Endocrinology And Metabolism*, 260(6), pp.E811-E824.

Watt, M.J. and Hargreaves, M. (2002). 'Effect of epinephrine on glucose disposal during exercise in humans: role of muscle glycogen'. *American Journal of Physiology, Endocrinology and Metabolism.* 283(3), pp. E578-E583.

Widrick, J.J., Costill, D.L., Fink, W.J., Hickey, M.S., McConell, G.K. and Tanaka, H. (1993). 'Carbohydrate feedings and exercise performance: effect of initial muscle glycogen concentration'. *Journal of Applied Physiology*, 74(6), pp.2998-3005.

Williams, C., Nute, M.G., Broadbank, L. and Vinall, S. (1990). 'Influence of fluid intake on endurance running performance. A comparison between water, glucose and fructose solutions'. *European Journal of Applied Physiology and Occupational Physiology,* 60(2), pp.112-119.

Wojtaszewski, J.F., MacDonald, C., Nielsen, J.N., Hellsten, Y., Hardie, D.G., Kemp, B.E., Kiens, B. and Richter, E.A. (2003). 'Regulation of 5′ AMP-activated protein kinase activity and substrate utilization in exercising human skeletal muscle'. *American Journal of Physiology-Endocrinology and Metabolism*, 284(4), pp.E813-E822.

Wright, D.A., Sherman, W.M. and Dernbach, A.R. (1991). 'Carbohydrate feedings before, during, or in combination improve cycling endurance performance'. *Journal of Applied Physiology,* 71(3), pp.1082-1088.

Yeo, W.K., McGee, S.L., Carey, A.L., Paton, C.D., Garnham, A.P., Hargreaves, M. and Hawley, J.A. (2010). 'Acute signalling responses to intense endurance training commenced with low or normal muscle glycogen'. *Experimental Physiology,* 95(2), pp.351-358.

Yeo, W.K., Paton, C.D., Garnham, A.P., Burke, L.M., Carey, A.L. and Hawley, J.A. (2008). 'Skeletal muscle adaptation and performance responses to once a day versus twice every second day endurance training regimens'. *Journal of Applied Physiology,* 105(5), pp.1462-1470.