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Delaying consumption of a carbohydrate-rich breakfast does not impair afternoon intermittent exercise performance.

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Abstract

Background: Omission of a carbohydrate-rich breakfast has been shown to impair afternoon/evening exercise performance, but previous studies have been limited by a lack of placebo control and the inclusion of complete omission of feeding until lunch, versus delaying the breakfast feeding. In this randomised, single-blind, placebo-controlled study, we hypothesised that introducing a placebo control would show no-difference to prolonged intermittent exercise performance in the afternoon versus consuming a high-carbohydrate breakfast. **Methods:** Ten regular intermittent games players completed two trials (EARLY and DELAY) that were matched for energy intake. In EARLY, participants consumed a high-carbohydrate breakfast shake (2 g·kg BM^{-1} maltodextrin, 1 ml·kg BM^{-1} orange squash, 0.15 g·kg BM^{-1} Xantham gum, 0.067 g·kg BM^{-1} artificial sweetener and 6 ml·kg BM^{-1} water) at 8am, followed by a taste and texture matched, but energy depleted, placebo (Identical minus Maltodextrin) at 10am. In DELAY the order of these shakes was reversed. In both trials, a standardised and individualised high carbohydrate lunch (888±107 Kcal, 145±28 g carbohydrate) was consumed at 12pm. Blood glucose and substrate oxidation measurements were conducted hourly throughout the day, and a subjective appetite rating taken after each meal. At 3pm, participants subsequently completed an 80-min intermittent exercise performance task, consisting of two, 40-min stages with 10-min of rest in between. Peak, mean, and end power output were measured during each sprint and averaged across each stage of the test for statistical analysis, with heart rate and RPE measured after each sprint. **Results:** The subjective appetite response followed a similar pattern during the morning of both trials, despite differing blood glucose and substrate oxidation results, which together confirmed the success of the single-blind placebo control. There were no differences in peak power (1st half: mean difference [95% CI]: 0.85 [-12 to 14] W, p=0.89, d=0.01); 2nd half: 1.6 [-12 to 15] W, p=0.79, d=0.01), mean power (1st half: mean difference: 2.2 [-12 to 17] W, p=0.73, d=0.01); 2nd half: mean difference: -2.2 [-16 to 11] W, p=0.72, d=0.02) or end power (1st half: mean difference: 6.9 [-11 to 25], p=0.42, d=0.05); 2nd half: mean difference: -1.7 [-16 to 12] W, p=0.80, d=0.01) in the DELAY compared to the EARLY condition. **Conclusions:** These data provide differing results to studies which focused on overt breakfast omission, rather than delaying breakfast using a placebo. This study provides preliminary suggestions that the results in other studies may likely be from a psychological, rather than physiological, mechanism.

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s).

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Introduction

Alongside genetics and exercise training, nutrition has long been recognised as one of the most powerful determinants of human physical performance, with early laboratory studies linking low carbohydrate availability and subsequent systemic hypoglycaemia to the onset of fatigue during exercise (Christensen & Hansen, 1939). Since this early work, our understanding of nutrition, and what is required nutritionally prior to and during exercise to optimise athletic performance, and after exercise to support recovery, has improved considerably. Nonetheless, despite significant scientific debate and research (Bergström et al., 1967; Burke, Ross, et al., 2017; Butki et al., 2003; Hawley & Leckey, 2015; Krogh & Lindhard, 1920; O'Keeffe et al., 1989; Shaw et al., 2019), and with some specific exceptions such as restricting carbohydrate availability prior to exercise sessions to augment beneficial training adaptations (Cochran et al., 2015; Hansen et al., 2005; Hawley & Morton, 2014), the general rule that carbohydrates are the most important macronutrient to support performance has remained at the forefront of nutritional recommendations for athletes, particularly in sports where high-intensity exercise is crucial to performance.

Nutrition and nutritional advice for athletes revolve around three key pillars: the type, the dose required, and the timing of consumption. As referenced above, despite scientific debate, carbohydrate is well researched and accepted as the most important nutrient for exercise performance, and therefore questions around type are mainly answered by carbohydrate (and not fat) remaining king when related to exercise performance (Close et al., 2016). Our understanding of the dose needed at various times pre, during and post exercise are also well established through years of research, with evidence-based recommendations for the pre-exercise meal, post-exercise feeding, and the optimal level of carbohydrates to consume during exercise. Research also supports recommendations for the optimal dose of carbohydrate, delivered in g per kg, in the days leading up to exercise to promote maximal glycogen availability in the active skeletal musculature (Kerksick et al., 2018). However, within certain contexts, such as the pre-exercise meals on the day of exercise, less is known regarding the optimal timing of consumption to maximise performance, particularly if the exercise is taking place later in the day and therefore it is expected multiple meals would be consumed prior to the exercise taking place (Metcalfe et al., 2021). Indeed, current guidelines and recommendations primarily focus on the 1–4-hour window prior to the exercise, with only generic guidelines to consume a diet rich in carbohydrate in the lead-up to an exercise bout other than this. When considering athletes who compete in the afternoon and/or evening, this potentially misses a large window of opportunity in the morning and/or early afternoon; a window where the timing of nutritional intake may be increasingly important in a sporting sphere where marginal gains can make the difference.

This hypothesis has in recent years began to be supported by a developing body of research which has investigated the effects of eating vs skipping breakfast on afternoon and/or evening performance, which thus far has collectively provided tentative evidence that the consumption of a high carbohydrate breakfast enhances, or conversely the omission of a high carbohydrate breakfast impairs, subsequent exercise performance in the afternoon/evening period (Bin Naharudin et al., 2019; Clayton et al., 2015; Cornford & Metcalfe, 2018; Metcalfe et al., 2021). However, up to the present no study has investigated this research area against a control, or placebo control, despite studies in other similar areas of sport science (e.g. breakfast and immediate exercise performance) showing that a placebo control can affect the study outcome (Mears et al., 2018; Naharudin et al., 2020). The current thesis will therefore build on the current body of work, providing novel and innovative findings, by investigating the effects of delaying the consumption, rather than complete omission as in previous studies, of a high carbohydrate breakfast upon subsequent afternoon intermittent performance by using a placebo control. By delaying the consumption of breakfast, the aim is to better reflect an athlete’s eating habits on a matchday, contributing to a greater understanding of the school of thought associated with the current thought process that the consumption of breakfast is important for evening exercise performance. A further aim is to explore the effects of using a mode of exercise commonly played during the afternoon/evening period, and the impact nutritional timing could have on performance in prolonged intermittent exercise. The aim of the following literature review is therefore four-fold: 1) to provide an overview of CHO metabolism; 2) to critically review the literature on the effects of CHO prior to, during, and in recovery from exercise; 3) to critically review the currently available literature on breakfast and afternoon and evening performance; and 4) synthesise the current state of the literature to provide a justification and rationale for the research study and hypothesis to be explored in this thesis.

Literature Review

An Overview of Carbohydrate Metabolism

With the importance of carbohydrates to exercise performance well known, the mechanisms of how they reach the active musculature are pertinent to the preceding review. Metabolism of complex carbohydrates begins in the mouth, where the salivary enzyme amylase converts the dietary polysaccharides into maltose molecules, before they subsequently travel to the small intestine, where further enzymes degrade disaccharides (namely lactose, sucrose, and maltose) into their constituent monosaccharides. Following this, active or passive diffusion pathways are utilised to allow absorption from the intestinal epithelium (Dashty, 2013). Passive diffusion occurs with the phosphorylation of the monosaccharide leading to their facilitated transfer into the circulation through glucose transporter (GLUT) proteins, before being de-phosphorylated and entering the liver. The active diffusion pathway involves the use of a sodium glucose linked transporter protein (SGLT) with an energy coupled mechanism (Navale & Paranjape, 2016).

There is a relatively small amount of glucose circulating in the blood (approximately 4 grams) and blood glucose concentrations must be kept within narrow limits (4-7 mmol/L) to remain healthy, and therefore excess energy intake in the form of glucose after feeding is rapidly taken up and converted/stored as glycogen, mainly in the skeletal muscles or the liver. For skeletal muscle, this process occurs via insulin-stimulated glucose disposal, with insulin stimulating the translocation of GLUT-4 transporters to the cell membrane via vesicular transport, which subsequently allow for the facilitated diffusion of glucose into the muscle cell, allowing blood glucose homeostasis to be maintained (Stöckli et al., 2011). Once glucose has been transported into the cell, it is either synthesised to become glycogen and subsequently stored in the muscle cell, entered into the glycolysis pathway to be oxidised into pyruvate which then enters the TCA cycle and is eventually converted to ATP (Gromley & Gromley, 2019), or facilitates substrate level phosphorylation in the cytosol and maintains the downhill gradient for glucose metabolism (Müller et al., 2018).

Glucose can also be stored in the liver, where it is transported insulin-independently directly from the small intestine via the portal vein and absorbed into the liver through GLUT-2 transporter proteins, before being phosphorylated into glucose-6-phosphate, to maintain the concentration gradient needed for continued facilitated diffusion of carbohydrates (Adeva-Andany et al., 2016). During a fasting period, such as the overnight fast, glucagon levels increase above insulin levels, causing tissues to transition to alternative fuels as a means of maintaining glucose homeostasis. This causes an increase in fatty acid oxidation by the skeletal muscle and liver, whilst the livers primary role in this state is to synthesise and release glucose through gluconeogenesis and glycogenolysis respectively to maintain blood glucose levels within the required parameters (Gromley & Gromley, 2019).

Carbohydrates & Exercise Performance

Carbohydrates, and specifically glucose, are arguably the most important nutritional element, as a ubiquitous source of energy for all living organisms. Once entering the body glucose is delivered to energy requiring tissues, such as the skeletal muscle which begins the process of cellular respiration, eventually forming adenosine triphosphate, which can provide an energy substrate for almost every bodily function through a series of biochemical reactions (Hantzidiamantis & Lappin, 2022). Carbohydrates are important substrates for exercise, as they enable skeletal muscle contraction through ATP resynthesis, with skeletal muscle fatigue correlating strongly with depletion of carbohydrate reserves, albeit carbohydrate utilisation can vary depending on a number of factors such as exercise intensity and duration, environment and gender (Hargreaves, 1991). During exercise, muscle contraction is initiated by calcium ions being released from the sarcoplasmic reticulum and into the sarcoplasm, where they bind to troponin, maintaining the unshielded actin binding site, allowing myosin to bind and cross bridge cycling to occur (Betts, 2013). ATP drives the activity of key enzymes involved in membrane excitability, sarcoplasmic reticulum handling and cross-bridge cycling, with glucose and fat mobilised to meet this high ATP demand in the muscles as a result, with glucose favoured during higher intensity exercise (Hargreaves & Spriet, 2020). The mechanism of mobilisation has not been well researched in human muscle; however multiple studies have used mice or rats, due to their mammalian muscles. The prevailing concept is that mobilisation of glucose is initiated from metabolic feedback signals through the AMP activated Protein Kinase (AMPK) signalling pathway, and mechanical stress related signalling cascades (Jensen et al., 2014).

The body's ability to store carbohydrate is relatively small and stores of carbohydrate can be acutely manipulated by a single meal or exercise bout (Costill, 1988). As a result, to avoid both hyperglycaemia and a loss of glucose through the urine, carbohydrates are (after being broken down into monosaccharides, i.e., glucose) rapidly transferred into cells and converted into glycogen, which is stored and ready to be mobilised according to activity demand (Björntorp & Sjöström, 1978), or transported directly to the liver from the intestine to be converted to glycogen and stored, with any excess after stores are full also being diverted to the liver to be converted into adipose via de novo lipogenesis (Adeva-Andany et al., 2016). The major sites of carbohydrate storage as glycogen are in the liver and skeletal muscles, and with the muscular system accounting for 20-30% of bodyweight, glycogen stores in the muscle are much higher than in the liver, with approximately 500 g of glycogen stored in the skeletal muscle compared to approximately 100 g in the liver, although the concentration within each muscle cell is very small (Sherman, 1995; Sherman & Wimer, 1991). As a result, glycogen concentrations in the liver are much larger than in any individual skeletal muscle, due to the difference in size. Macauley and colleagues demonstrated this, showing glycogen concentrations in the liver after an overnight fast to be 325.9 mmol/L, versus only 68.1 mmol/L in a fasted gastrocnemius muscle, with concentrations further increased after a high-CHO meal containing 120 g carbohydrate, by around 19% in the liver (388.1 mmol/L) versus 17.3% (79.7 mmol/L) in the muscle (Macauley et al., 2015). During exercise, there is a rapid breakdown of this glycogen to enable continued muscle contraction and ensure that

ATP demand is matched by ATP synthesis. This occurs through the activation of phosphorylase kinase, which transforms glycogen phosphorylase into its more active form through covalent regulation. From here, phosphorylase A breaks down glycogen and its products combine with an inorganic phosphate, producing glucose-1-phosphate, glucose-6-phosphate, and fructose-6-phosphate in the glycolytic pathway, which continues to produce a net 3 molecules of ATP and lactate formation (Hargreaves & Spriet, 2020). This all occurs in a cyclic nature in milliseconds, and studies have shown the effect this has on glycogen stores in the muscle. In one study, after only a single 6 second bout of high-intensity exercise, muscle glycogen levels in the vastus lateralis were reduced by 14% (Gaitanos et al., 1993). Although the level of glucose utilisation depends on the exercise intensity. At lower intensities, glycogen breakdown will be relatively low with the contribution from fat. However, as exercise intensity increases, glycogen breakdown becomes increasingly important, with a study showing total body fat oxidation was decreased by 34% at an exercise intensity of 75% Wmax, versus at 55% Wmax (Van Loon et al., 2001).

Research focusing on the effects of carbohydrate feeding on exercise performance and showcasing its beneficial properties have been available for over a century, with the earliest studies showing that participants reported exercise to be easier if consuming a carbohydrate rich diet versus a fat rich diet amid regular exercising (Krogh & Lindhard, 1920). Studies in the 1960's advanced the knowledge further using the muscle biopsy technique to show the effects of muscle glycogen, linking participants improved exercise performance after consuming a high-carbohydrate diet to increased concentrations of glycogen in the muscle (Bergström et al., 1967; Bergström & Hultman, 2009). Studies since then have continued to evidence the prevailing benefits of carbohydrate to exercise performance, despite the promotion of alternative nutritional diets, such as ketogenic diets. Ketogenic diets promote the oxidation of fats by reducing dietary carbohydrate consumption to a level that incurs nutritional ketosis, resulting in a diet that is composed of almost no carbohydrate (Volek et al., 2015). A study by Burke et al. (2017) however provided evidence to suggest that a ketogenic diet does not improve, or even maintain, performance versus a high-carbohydrate diet, by showing that elite race walkers consuming a low carbohydrate/ketogenic diet for three weeks of intensified training prior to a 10km elite race walk reduced their race walk time by 1.6% and exhibited increased oxygen demand compared to the high CHO diet, who were able to improve by 6.6%. The increased oxygen cost of ATP production from fat vs carbohydrate seemed to negate any training induced increase in peak aerobic capacity and limits exercise capacity (Burke, Ross, et al., 2017), providing further evidence that carbohydrate remains the key nutritent when it comes to optimising exercise performance. It is clear then, that there is a large body of evidence now that shows that regardless of the exercise mode, exercise length, or exercise intensity, carbohydrate remains a key element to ensure optimised performance, and these studies will be discussed in detail further on in this review. Without sufficient fuelling of carbohydrate in the days prior to, on the day of and throughout the completion of an exercise bout, there will be a lack of carbohydrate availability to the active skeletal muscle during exercise and this will cause fatigue will set in more rapidly, ultimately negatively affecting exercise performance (Costill & Hargreaves, 1992). Taken all together, the importance of carbohydrate, both before and during exercise performance is clear.

The recommendations for carbohydrate intake can generally be split into three main sub-sections: In the days prior to an event, on the day of the event, during the event and in the period after the event. The subsequent sections of the review will focus on each of these contexts in turn.

Carbohydrates prior to the day of exercise

In the days prior to an event/match/competition, there are differing strategies based on the demands of the sport, all with the goal of ensuring high carbohydrate availability during exercise, as carbohydrate availability is a limiter to performance in prolonged sub-maximal or intermittent exercise and sustained high intensity work (Burke et al., 2011; Hargreaves, 1999). A commonly used and heavily researched strategy for ensuring carbohydrate availability in the days prior to exercise performance is that of ‘carbohydrate loading’, which is most often reserved for continuous and/or endurance athletes completing exercise for more than 90 minutes, due to evidence suggesting that glycogen loading has no impact on performance when the bout is less than 90 minutes (Hawley et al., 1997). Carbohydrate loading, originally derived by a Scandinavian research team, involves a protocol which enables glycogen supercompensation, which is achieved initially through a period of muscle glycogen depletion a week prior to the event, where the athletes elevates their normal workload but does not match this with carbohydrate consumption, instead ingesting a low amount of carbohydrates, followed by a further 3 day loading phase up until the event day, whereby exercise volume and intensity is tapered but carbohydrate intake is high (Karlsson & Saltin, 1971). However, more recent research has shown that there is actually little to no benefit of the depletion phase where restricting carbohydrate intake or exercising at a greater volume/to exhaustion occurs, and thus a modified protocol was created by Sherman et al., (1981). This protocol asks the athlete to taper down exercise volume/intensity for consecutive days, before not exercising at all on the day before competition, whilst concurrently consuming a mixed diet for 3 days followed by a high-CHO diet for the following 3 days (Sherman et al., 1981). However, this regimen has had low adherence in practice as whilst it removes the depletion phase, overall, it is more time consuming for the athlete (6-d) and in fact, a study has more recently shown that just a single day of high carbohydrate consumption can increase muscle glycogen stores by 90% when coupled with physical inactivity on that day, with no further benefits after two more days of the intervention (Bussau et al., 2002). However, there are still issues around the strategy of carbohydrate loading, as research has more recently found that multiple negative connotations can be attached to both the low carbohydrate intake and glycogen depleting exercise when only 3-6 days out from an endurance event. Low carbohydrate intake can induce adverse physical and mental health consequences, whilst glycogen depleting bout followed by little to no exercise in the 3 days before an event can severely disrupt an athlete's rhythm, causing adverse performance regardless of carbohydrate intake (Sedlock, 2008). Further concerns include higher risk of injury due to a lack of glycogen stores and carbohydrate availability, which inadvertently cause irritability, diminished mental acuity and listlessness because of the effects of hypoglycaemia (Jeukendrup et al., 2005; Sedlock, 2008). It is clear that when considering this strategy prior to an endurance event, there are other factors that should be considered. These can include (but are not an exhaustive list) the loading strategy selected, the type of carbohydrate consumed, the exact characteristics of the tapered exercise performed, the timing of CHO ingestion each day, and gender (Sedlock, 2008).

Concerning the type of carbohydrate to consume, it is speculated that a mix of different monosaccharides would be most useful, particularly for liver glycogen repletion as liver stores can become severely depleted in the postabsorptive phase (Podlogar & Wallis, 2022). Multiple studies have shown that combining glucose-based carbohydrates with fructose or galactose improves liver but not muscle glycogen synthesis, versus glucose based carbohydrates alone (Décombaz et al., 2011; Detko et al., 2013; Fuchs et al., 2016). Overall, athletes should choose compact, complex starchy carbohydrate sources (Evans & Hughes, 1985) containing a mixture of glucose and other monosaccharides, that are low in fibre and easy to consume in order to reduce gastrointestinal discomfort whilst still meeting goals for a high-carbohydrate diet, with the dose being individualised and set in grams per weight in kilograms per day (Burke et al., 2011). This may include pasta or rice, whilst another important consideration may be utilising a carbohydrate-electrolyte beverages to meet the high carbohydrate goal whilst not adding too much 'bulk' to the diet, as this could pose problems with adherence and gastrointestinal discomfort (Sedlock, 2008). Due to its popularity and effectiveness in improving long duration sub-maximal performance, the topic of glycogen supercompensation continues to provide new potential strategies on a regular basis, and as an athlete and/or practitioner for an athlete, it is best to try and choose a strategy that is the most 'user-friendly' to the athlete. This may involve exploring differing strategies over a period of competitions/events and choosing the one which ultimately impacts upon pre-event preparation and/or exercise/training schedules the least. The basic recommendation to continually replace depleted muscle glycogen stores during training periods by consuming a diet high in carbohydrate still remains important, even with evidence and research around periodisation strategies that may suggest continuous high-carbohydrate diets are not always needed, depending on the exercise performed.

Carbohydrates on the day of exercise

The importance of feedings in the immediate hours leading up to exercise, or the pre-match meal as it is commonly referred to (i.e., food consumed within the 3–4-hour pre-match window) has been well researched in multiple modes of exercise. The ingestion of a high-carbohydrate meal has been shown to improve performance when compared to a placebo/no carbohydrate ingestion, whilst total work produced was 18.2% greater in a 15-minute performance ride on an isokinetic cycle ergometer following a 45-minute cycle at 70% of $\dot{V}O_2$max when a high-carbohydrate meal was consumed 4 hours prior to exercise beginning vs a placebo (Neufer et al., 1987). Meanwhile, a high-carbohydrate meal (2.5 g/kg/bodyweight) consumed 3 hours prior to exercise was found to enhance endurance running capacity in a treadmill test to exhaustion at 70% $\dot{V}O_2$max by ~9%, with this increased to ~12% when a carbohydrate-electrolyte beverage was consumed during the trial in comparison to a placebo and water beverage (Chryssanthopoulos et al., 2002). The same results are found when completing resistance exercise, with Bin Naharudin et al., (2019) prescribing either a breakfast containing 1.5 g/kg/bodyweight, or no meal at all. The research found that when performing four sets to failure of the back squat and bench press at 90% of participants 10 repetition max, in the breakfast omission trial participants were able to perform 15% less back squat and 6% less bench press repetitions before failure than those who consumed breakfast, although it was acknowledged that due to the nature of the study, the participants knowledge that they were/were not consuming breakfast and the fact that all participants were habitual breakfast eaters may have caused a placebo effect to enhance performance in the breakfast consumption trial.

Indeed, the benefits of mixing multiple monosaccharides have also been found to extend to exercise performance, with a recent study finding cycling endurance exercise capacity was improved with the addition of fructose to a high-CHO breakfast, although further research is needed to determine the optimal dose and ratio (Podlogar et al., 2022).

Carbohydrates during exercise

To optimise carbohydrate fuelling strategies and ultimately further improve athletic performance, the introduction of the ingestion of carbohydrate during exercise has become common, to offset liver glycogen depletion that occurs when completing prolonged exercise bouts. The most common and well evidenced method to achieve this is using carbohydrate-electrolyte beverages or carbohydrate gels. Research has shown that carbohydrate ingestion can prolong time to exhaustion when exercising at a constant power output (Coyle et al., 1983, 1986; Tsintzas et al., 1996), improve high-intensity exercise performance and endurance capacity, even after a bout of prolonged continuous or intermittent exercise (Hargreaves et al., 1984; Nicholas et al., 1995), and improve road race times in moderate to high environmental temperatures (Millard-Stafford et al., 1992; Tsintzas et al., 1993). The mechanistic rationale behind these improvements in exercise performance is that the ingestion of carbohydrates, which are able to be quickly digested and mobilised to the active skeletal muscles, could either prevent systemic hypoglycaemia and/or promote glycogen 'sparing', whereby liver glycogen levels are not needed to be utilised at such a rapid rate and the ingested carbohydrate solution is able to be quickly utilised in the active musculature (Tsintzas & Williams, 1998). The benefits of including carbohydrate ingestion (a 6.9% carbohydrate-electrolyte gel) during long bouts of exercise, alongside the consumption of a high carbohydrate meal (2.5 g/kg/bodyweight) 3 hours prior to exercise, as per the current recommendations (Kerksick et al., 2018), has been shown to bring about a 22% greater endurance running capacity compared to a placebo, with the gel providing a further 12% improvement on top of consuming the meal alone, which itself was shown to improve running capacity by 9% compared to the placebo (Chryssanthopoulos et al., 2002).

To further maximise the performance benefits of ingesting carbohydrate during the exercise, the type of carbohydrate being consumed has been investigated. Interestingly, it has been established that different types of carbohydrate can be oxidised at different rates within the skeletal muscle due to the use of different transporter proteins at the brush border of the small intestine, thus the use of a combination of carbohydrate sources that use different transporter proteins, provide a quicker carbohydrate delivery and an increased oxidation rate, to further help the athlete (Jeukendrup, 2010). Compared with glucose only, ingesting a glucose and fructose mixture has been shown to improve time to completion of an endurance performance by 8% in a 120-minute cycling exercise at 55% Wmax followed by a time trial in which subjects had to complete a set amount of work as quickly as possible (Currell & Jeukendrup, 2008). The reason for this improvement in performance is due to improved rates of exogenous carbohydrate oxidation, which promotes sparing of muscle and liver glycogen and likely led to greater maintenance of hepatic glucose output later into the exercise performance (Currell & Jeukendrup, 2008). The enhanced carbohydrate delivery occurs in combination with increased fluid delivery, helping to further delay dehydration by enhancing gastric emptying and therefore fluid delivery to the active skeletal musculature (Jeukendrup & Moseley, 2010), also preventing the likelihood of gastrointestinal problems, which is a commonly noted problem amongst endurance athletes (de Oliveira & Burini, 2014). Research has shown the use of carbohydrate gels and beverages to be the most useful strategy to maintain blood glucose levels and attenuate endogenous muscle glycogen depletion and central fatigue (Saunders, 2007). Therefore, the recommendations when high intensity exercise bouts (working above 70% $\dot{V}O_2max$) exceed 90 minutes is to aim to consume 30-60 g of multiple transferable carbohydrates, such as glucose-fructose, per hour of exercise, ideally in a 6-8% carbohydrate-electrolyte beverage (Kerksick et al., 2017) to help achieve the benefits outlined above.

Carbohydrates after exercise

It is equally important for physical performance to ensure adequate carbohydrates are consumed after an exercise bout. The restoration of muscle and liver glycogen is a fundamental goal post exercise, which is all the more important if there is a short time period between events (Burke et al., 2011). Indeed, research shows that low/sub-optimal energy and carbohydrate intake in the 24 hours after a glycogen depleting event or match can compromise an athletes ability to train for up to 72 hours (Abreu et al., 2021; Burke, Van Loon, et al., 2017). Further studies show if carbohydrate ingestion is left for just two hours after exercise, muscle glycogen concentrations can be reduced by up to 45%, compared to carbohydrate feeding immediately after the cessation of exercise (Krustrup et al., 2006). This subsequently provides incomplete recovery and potentially limits performance capacity, particularly if the following exercise bout occurs soon after, as is commonplace in intermittent sports such as football (Kerksick et al., 2017). Muscle glycogen depletion is heavily associated with an increased injury risk, as a result of the onset of fatigue occurring earlier through the accumulation of intramuscular hydrogen ions and/or deprotonated phosphate, as well as the depletion of calcium and intramuscular substrates, of which muscle glycogen is one (Schlabach, 1994). Therefore, it is widely recommended that the athlete begin carbohydrate refuelling as soon as possible after the exercise bout and at regular and frequent intervals, particularly if the next exercise bout will be performed within less than 8 hours, as the glycogen synthesising enzymes are most active immediately after exercise (Ranchordas et al., 2017). The glycogen resynthesis after glycogen depleting exercise consists of two phases, which must be considered when implementing strategies to maximise recovery. The first is a rapid phase of glycogen resynthesis, lasting around 30-60 minutes, occurs without the presence of insulin and is instead caused by exercise-induced translocation of a glucose transporter protein to the muscle cell membrane, increasing the cells permeability to glucose. From here, in a phase that can last a few hours, glycogen synthesis rates are much slower, with both insulin and muscle contraction showing an ability to increase the activity of glycogen synthase, the rate limiting enzyme in glycogen synthesis (Jentjens & Jeukendrup, 2003). The importance of immediate carbohydrate consumption post glycogen depleting exercise is therefore imperative to maximise recovery, with glycogen synthesis rates reported to be highest (between 40 and 43 mmol/kg dw/h), when high levels of carbohydrates (1-1.85 g/kg/hr) are consumed immediately after exercise and at 15-60 minute intervals thereafter, with this effect remaining for up to 5 hours (Roy & Tarnopolsky, 1998; Van Loon, Saris, Kruijshoop, et al., 2000).

It is clear then that the timing of carbohydrate intake holds the highest importance in ensuring muscle glycogen stores are maximally and rapidly restored. A refuelling strategy of 0.6-1 g/kg/bodyweight within 30 minutes of a glycogen depleting exercise ending, and every two hours following this for between four and six hours, has been shown to promote maximal glycogen replenishment (Jentjens & Jeukendrup, 2003), whilst other studies have also shown similarly beneficial outcomes when 1.2 g/kg of carbohydrate were consumed every 30 minutes over a period of 3.5 hours (Van Loon, Saris, Kruijshoop, et al., 2000). Meanwhile, guidelines recommend a dosage of 1-1.5 g/kg/hour for the first 4 hours following a match, split into several meals, snacks and beverages consumed regularly throughout the 4 hours, and to avoid large meals and the associated gastrointestinal discomfort (Abreu et al., 2021; Burke et al., 2004). Based on the upper limit (1.5 g/kg/hour) of this recommendation, therefore, an 80 kg athlete should consume ~120 g per hour in the first few hours following exercise, with particular emphasis on achieving this target during times where the athlete will be required to compete again in a shorter space of time (Ranchordas et al., 2017).

However, whilst there are advantages to small and frequent ingestion of carbohydrates in the short term recovery period (first 4-6 hours), over a longer recovery period (24 hours) carbohydrate intake should be organised into a pattern of carbohydrate-rich meals, consumed with even spacing, or as even as possible with regard to individual athletes situations and needs (Burke et al., 2004; Kerksick et al., 2017). The carbohydrates consumed during the initial 6-hour recovery period should have a moderate to high glycaemic index as these provide a sufficiently elevated increase in blood glucose and insulin concentrations versus lower glycaemic index carbohydrates (Abreu et al., 2021; Collins et al., 2021). The importance of insulin in enabling endogenous carbohydrate storage is also well established, and the elevated levels of insulin in the blood in response to high glycaemic index carbohydrates versus low glycaemic index carbohydrates provides the opportunity to further improve glycogen resynthesis post-match/competition (Betts & Williams, 2010).

It is accepted, however, that it may not always be feasible to consume this level of carbohydrate immediately following exercise and certain acute strategies have emerged which can aid or accelerate muscle glycogen synthesis, namely co-ingesting carbohydrate with protein. Given that protein is also an essential part of an athlete’s recovery due to its role in supporting skeletal muscle and whole-body adaptations, co-ingestion along with carbohydrates seems to bring about multiple benefits when considering optimal recovery (Abreu et al., 2021). The mechanism behind this strategy lies in the ability of free amino acids to induce pancreatic secretion of insulin which in turn promotes greater uptake of glucose from the blood into muscle cells (Betts & Williams, 2010). A mixture of whey protein hydrolysate and free amino acids, ingested orally at a rate of 0.4 g/kg/hour alongside a glucose: fructose carbohydrate solution ingested at a rate of 0.8 g/kg/hour, has been found to induce an insulin response 88% higher than if the carbohydrate solution was consumed alone, over a 5 hour period (Van Loon, Saris, Kruijshoop, et al., 2000). Further research identified the best amino acid mixture to promote secretion of insulin from the β-cells of the pancreas was leucine and phenylalanine, which when mixed with whey protein hydrolysate and carbohydrate caused a 103% increase in the insulin response versus the carbohydrate only drink (Van Loon, Saris, Verhagen, et al., 2000). There was a strong positive correlation between this insulin response and levels of plasma leucine, phenylalanine, and tyrosine, providing evidence of the insulinotropic response these free amino acids can provide (Van Loon, Saris, Verhagen, et al., 2000). The correlation with tyrosine, despite it not being present in the drink, may involve the fact that tyrosine is formed by the hydroxylation of phenylalanine and should be noted as another amino acid which provides a positive insulin response yet does not provide the athlete with a negative gastrointestinal response as other amino acids have been shown to, such as arginine (Van Loon, Saris, Kruijshoop, et al., 2000; Van Loon, Saris, Verhagen, et al., 2000). It is recommended that protein be co-ingested alongside carbohydrate at a rate of 0.3-0.4 g/kg/hour to maximise the insulinotropic effect, which is also the optimal amount necessary to promote muscle protein synthesis, hence is doubly beneficial for recovery (Burke, Van Loon, et al., 2017). However, it is important to note that adequate carbohydrate intake negates any benefit of co-ingestion from a muscle glycogen standpoint, as when carbohydrate intake post-event meets the recommendations, co-ingestion with protein has no further effect on glycogen synthesis (Betts & Williams, 2010). Although, given the evidence that some professional athletes, particularly female athletes, and football players, often do not meet the guidelines for optimal carbohydrate consumption (García et al., 2014; Lun et al., 2009; Macuh et al., 2023), protein co-ingestion could be seen as an important strategy that can be utilised for a practical way to ensure athletes aim to meet the necessary muscle glycogen repletion rate to maintain optimal performance.

With reviews continuing to highlight the dependence on a high carbohydrate diet for athletes aiming for success in endurance events and team sports (Cermak & van Loon, 2013; Kerksick et al., 2018; Williams & Rollo, 2015), much of the research focus is shifting onto the optimal dosage and type for carbohydrate intake in these athletes. Naturally, due to the differing demands of sports and athletic events, a “one size fits all” approach does not work, and sport-specific goals should always be favoured, i.e. a marathon runner will need to consume more carbohydrate than a sprinter for instance, but the most important facet of any recommendation is that carbohydrate should form an essential component of all athletes’ diets. As a general rule, it is recommended that, in order to replenish endogenous muscle glycogen stores, an athlete should continually consume a diet that contains 5-12 g of carbohydrate per kg of bodyweight per day, with a particular focus on the higher end of this range when completing high volumes (>12 hours per week) of intense exercise (>65-70% $\dot{V}O_2max$) as this is when muscle glycogen stores will become most depleted (Kerksick et al., 2017).

Carbohydrates and Intermittent Exercise Performance

Intermittent sports, such as football, rugby, netball etc, are characterised by the repetition of several diverse activities, such as walking, jogging, sprinting and changes in direction requiring rapid and efficient deceleration and subsequent acceleration (Bangsbo et al., 1991). Time-motion analysis during football has shown that there is a change in the intensity of the activity every 4-6 s, meaning that a player at the top level of their sport completes around 1350 unique activities per game, not including any game specific energy demands such as jumping, dribbling etc (Mohr et al., 2003). As is the nature of fatigue, more of this high-intensity work occurs in the first 15 minutes than the last 15 minutes, with work rate also decreasing in the second half regardless of competitive standard or position (Mohr et al., 2003). However, with the development of sport science support, and systemic training programmes, it is suggested that the fall in work rate will be less pronounced and allow for more direct comparison of performance metrics between halves (Reilly et al., 2008). Intermittent athletes are also required to be able to repeatedly produce maximal or near maximal sprints of short duration (1-7 s) with brief and incomplete recovery periods in between, which has been defined as “repeated sprint ability” (Rampinini et al., 2007). As such, the glycogen stores in the active musculature have repeatedly been shown to be a limiting factor to maintaining exercise performance at high intensities (Jacobs et al., 1982; Saltin & Karlsson, 1971), which accounts for the majority of the load in prolonged intermittent games (Reilly, 1997). Indeed, physiological and metabolic measurements, such as heart rate and blood lactate, taken during simulated football match play have shown that the average intensity exceeds the minimum threshold for much of the energy demand to be fulfilled by glycogenolysis (Jacobs et al., 1982).

Performance in intermittent sports, therefore, decreases due to repeated neuromuscular fatigue after repeated, intense brief efforts, manifesting as a reduction in the capcity of the skeletal muscle to generate force (Green, 1997). This may be from peripheral fatigue in the muscle, or central fatigue from the central nervous system, with studies showing that peripheral fatigue sets in earlier and manifests over the prolonged nature of intermittent exercise, whereas central fatigue tends to onset nearer towards the end of the task (Hureau et al., 2014, 2016) with the suggestion therefore that the fatigue mechanism for intermittent exercise are a combination of peripheral and central fatigue. However, research has shown that the increase in inorganic phosphate in the muscle, resulting from the breakdown of phosphocreatine (PCr) and adenosine triphosphate (ATP), correlates well with the aforementioned reduction in force capacity of the active musculature during repeated maximal contractions (Bogdanis et al., 1996), suggesting that it is peripheral fatigue that plays a greater role in the onset of fatigue versus central fatigue.

An investigation into the muscle and blood metabolites of professional soccer players also found that muscle glycogen reduced from 449 ± 23 pre-match to 255 ± 22 mmol.kg dry weight by the end of the game, with 47% of muscle fibres being completely or almost empty after the game (Krustrup et al., 2006). In another study, the quadriceps femoris muscle of recreational soccer players was biopsied at the beginning, half-time and full time of a match. Muscle glycogen concentrations were significantly lower at full time compared to pre-match, but importantly, those who started the game with lower muscle glycogen concentrations were reported to have almost fully depleted muscle glycogen stores at half-time of the game, whereas those who began with higher muscle glycogen levels exhibited much higher levels at half-time, and performed better physically during the game, covering a greater overall distance and completing a higher number of high intensity runs over the course of the game (Saltin, 1973). Together, it appears that fatigue during the latter stages of a game/match may in part be attributed to lower levels of muscle glycogen, but how low glycogen must be to negatively influence performance is unclear. One explanation is the attenuated glycogen concentrations resulting in a deficiency of the body's energetics, with the tricarboxylic acid cycle (TCA) unable to keep up with the rate of energy demand, which ultimately affects the muscles contractile processes (Bangsbo, 2000).

Athletes competing in intermittent activities do so at a high energy cost, due to the nature of their movements. Athletes who play intermittent team sports, such as soccer, rugby, and netball, need to consume an appropriate level of food that reflects their high levels of expenditure when training and playing in matches or competition. The estimated energy cost of an 80-90 minute game, for a player of average weight (75kg), is between 1519-1772 kcals (Stølen et al., 2005) based on an average exercise intensity of 85% HRmax and an average $\dot{V}O_2max$ of 75%, which has been shown by multiple pieces of research to be the average aerobic loading during a game (Bangsbo, 2000; Mohr et al., 2003; Reilly, 1997). This makes the provision of appropriate levels of carbohydrate on a match-day crucial for maximising glycogen reserves and ensuring players are physiologically prepared for competition. The focus of the pre-match meal is to consume easily digestible, low glycaemic index carbohydrates, with the goal to increase resting levels of muscle and liver glycogen (Rollo, 2014). Providing players with this high-carbohydrate meal 3-4 hours prior to intermittent games/competitions has been demonstrated to delay the onset of fatigue during a game and maintain performance levels for longer through the match (Reilly et al., 2008). If athletes do not consume a high-carbohydrate diet and therefore begin exercise with depleted glycogen stores, they cannot run as far as those who consume a high-carbohydrate diet, with a study finding a mean of 17% less distance covered overall, and over 75% covering more distance in sprints (>24.15km/h) (Souglis et al., 2013). Restricted/depleted carbohydrate diets cause lower levels of carbohydrate oxidation during exercise and subsequently higher free fatty acid oxidation, which whilst beneficial in prolonged moderate intensity exercise, is inhibitive to the supramaximal, intermittent type exercise most common in sports like football (Costill et al., 1977).

For intermittent exercise, such as football, it is therefore recommended that carbohydrate intake be within the range of 6-8 g/kg/day on a regular basis, i.e., throughout the week and leading up to the match (Abreu et al., 2021). This is because of training on the two days leading up to a match is still likely to be intense enough to deplete muscle glycogen stores. It has been recommended that the pre-match meal should be consumed 3-4 hours prior to the match beginning and should contain 2-3 g/kg/body mass, however literature shows that often players do not reach this figure and obtain less than 1.5 g/kg/body mass, which could affect players trying to reach optimal carbohydrate availability (L. Anderson et al., 2017). When investigating the effect of high vs low carbohydrate diets on intermittent exercise performance, specifically soccer, it was found that those consuming high carbohydrate diets, including a meal 3-hours pre-match, completed approximately 33% more high-intensity running than those consuming a low carbohydrate diet/meal (P. Balsom et al., 1999). The importance of carbohydrate consumed prior to and throughout multiple exercise modes, including intermittent exercise, is clear and well-defined in the literature.

When a high-carbohydrate meal is consumed pre-intermittent exercise, athletes have exhibited improve exercise performance through greater carbohydrate availabilty. In a 90-minute 4-a-side football game following a pre match meal containing either 65% carbohydrate or 30% carbohydrate, the concentration of muscle glycogen was significantly higher, and this translated to significantly more high-intensity exercise being performed during the game (P. Balsom et al., 1999). In a similar study utilising Gaelic football as the exercise performance, sprint performance was again significantly worse in the low carbohydrate diet, suggesting that having a high carbohydrate availability window can reduce declines in physical performance (O'brien et al., 2021). Further, in simulated rugby league matchplay, the coingestion of caffeine with a high-carbohydrate meal found that mean running speeds were likely to highly likely increased, along with sprint performance and high intensity running (Clarke et al., 2019). All the above studies link to the notion that the availability of carbohydrate in the active musculature is a key factor to intermittent exercise performance.

A less researched development for athletes competing within intermittent sports, but for which there is growing evidence, is the idea of carbohydrate periodisation. Carbohydrate periodisation revolves around the concept of "fuelling for the work required" and given the large amounts of data collection around training loads and how this fluctuates, it is a realistic possibility for intermittent sports like football to prescribe carbohydrate and energy intake to match the fluctuations in training load throughout the week leading up to a matchday. Indeed, it seems that this is in part already done by some athletes, potentially unconsciously. Research from Anderson et al. (2017) into the energy intake and expenditure of elite footballers showed that absolute carbohydrate intake and exogenous carbohydrate feeding is greater on a matchday than a training day. However, questions remain, with a review suggesting that carbohydrate periodisation does not seem to enhance performance versus simply consuming a high-CHO diet daily in endurance athletes (Gejl & Nybo, 2021). It may be that the “fuel for the work” paradigm has merit but further research should be done to determine its complete applicability, with studies now beginning to formulate contemporary guidelines that encompass carbohydrate periodisation (L. Anderson et al., 2022). Other research into fuelling strategies for intermittent athletes has shown that when co-ingesting protein and carbohydrate every 15 minutes during exercise, performance in an intermittent exercise test elicited a small, albeit not significant improvement (Highton et al., 2013), whilst a c trial using a 75 minute football-specific intermittent exercise test followed by a run to fatigue when ingesting a carbohydrate or carbohydrate-protein beverage prior to and at halftime of the test showed a significantly greater running capacity in the run to fatigue when co-ingesting the carbohydrate-protein beverage vs carbohydrate alone (Alghannam, 2011). Further research on this topic is needed to understand its viability for practitioners leading on fuelling strategies in intermittent sport.

Breakfast & Afternoon/Evening Performance

For athletes, breakfast is an important nutritional tool for performance, whether performance occurs early in the morning, during the day or even in the evening. By not eating breakfast, previous studies have shown that this risks cognitive declines and negative alterations to metabolism as a result of reduced carbohydrate availability to the brain (Pollitt, 1995). In comparison to this, when consuming breakfast athletes have shown an improvement in performance, because of the breakfast meal restoring liver and muscle glycogen levels after the overnight fast (Burke et al., 2004). It is useful though to define breakfast, with it being proposed that breakfast could be defined as “the first meal consumed within 2hrs of waking from the longest sleep in any 24hr period, that contains at least 50kcals” (Betts et al., 2016). Whilst there is a lack of recommendations on the contents of breakfast for athletes specifically, it is encouraged for athletes to consume breakfast regularly (Shriver et al., 2013) and in line with general recommendations for the maintenance of carbohydrate levels to meet and sustain the demands of exercise, whether that be training or competition (Collins et al., 2021; Kerksick et al., 2018).

Interestingly, whilst the beneficial effects of a pre-exercise high carbohydrate meal have been demonstrated, in the studies conducted to date this meal has exclusively been provided 1-4 hours prior to the exercise bout. Indeed, aside from a general recommendation to consume a high-carbohydrate diet to maintain, replenish or enhance skeletal muscle and liver glycogen availability, specific recommendations on competition-day dietary intake are focused on the 4 hour "pre-exercise" window (Kerksick et al., 2017). Although important, given that competitions/matches in many intermittent sports such as football, rugby and netball tend to start in the afternoon (3pm) or evening (e.g. 7-8pm), for athletes competing at these times, there is a far greater pre-exercise window (between 6-12 hours) in which nutrition could be manipulated/optimised in order to affect subsequent performance. It is therefore surprising that until recently very few studies have examined the effects of early morning dietary intake (e.g. breakfast) on subsequent afternoon/evening exercise performance. The research to date has provided preliminary evidence that skipping a high-carbohydrate breakfast may impair evening exercise performance (Clayton et al., 2015; Cornford & Metcalfe, 2018; Metcalfe et al., 2021). This comes against the backdrop of research raising concerns around athletes reporting skipping breakfast or eating a breakfast containing insufficient energy or carbohydrates to meet pre-exercise recommendations (Ruiz et al., 2005). Furthermore, in a small survey, a majority of fifty-two female collegiate athletes reported regularly skipping breakfast altogether, both generally and around game days (Shriver et al., 2013). However, in the study from Ruiz and colleagues, breakfast was not clearly defined, whereas Shriver and colleagues coded breakfast as "any caloric food/beverage consumed between 6:00 and 8:59 AM on each of the reported days". This differs to our studies definition of breakfast from Betts et al. (2016) and showcases the ongoing ambiguity and difficulty of accurately defining breakfast, which makes it difficult to directly interpret the findings of these studies in relation to this literature review and the current studies in the field of delaying and/or omitting breakfast. However, whilst the lack of a universally accepted definition of breakfast makes it difficult to make a direct comparison/interpret the findings of studies investigating breakfast effectively, the data collected still shows a lack of overall energy and nutrient intake amongst athletes against recommended benchmarks. This is important in the context of developing strategies to better ensure an athlete consumes appropriate and optimal nutrition ahead of any prolonged exercise performance.

Clayton et al. (2015) performed the first study of the effects of a high-carbohydrate breakfast on evening endurance exercise performance. In a randomised cross-over design, ten recreationally active male participants performed two laboratory based experimental trials where they either consumed a high-carbohydrate breakfast or extended their overnight fast until 12pm. The high carbohydrate breakfast meal contained approximately 25% of a participants estimated daily energy requirements (calculated using resting metabolic rate multiplied by a physical activity level of 1.7), which amounted to 3095 ± 195 kJ, with 11% derived from protein sources, 17% from fat and 72% of energy derived from carbohydrates. The meal was consumed at approximately 08:00am (0hrs). Participants were then asked to rest quietly until being allowed to consume an *ad libitum* lunch meal at 12:30 (4.5hrs) which was consisted of cold, ready to eat buffet style foods provided in excess of expected consumption. Participants consumption was measured by weighing the foods placed out before and after eating, with participants being given 30 minutes to eat as much as they wished. After further quiet rest, participants performed an endurance exercise test, beginning at 17:00 (9hrs). The exercise test consisted of a 30-minute steady state "preload" at approximately 60% $\dot{V}O_2$peak followed by a 30-minute stage at a self-selected intensity where participants were asked to complete as much work as possible. The key findings were that, although participants partially compensated for skipping breakfast by eating more at lunch, overall carbohydrate and energy intake prior to the exercise test were lower in the breakfast skipping trials compared to the breakfast consumption trial. In the breakfast trial participants performed a greater amount of work (~4.5%) compared to the breakfast skipping trial (Clayton et al., 2015).

Whilst the work from Clayton and colleagues provided novel evidence that skipping breakfast could have practical implications for optimising evening exercise training quality and performance during competition, it was unknown whether these findings persisted to a shorter duration, higher intensity exercise task. This question was addressed in a study by Cornford and Metcalfe (2018) who, in a randomised and counterbalanced crossover study, asked ten University level rowers to complete a 2000m rowing test in the evening after either consuming an individualised high-carbohydrate breakfast or extending the overnight fast until 12:00pm. *Ad libitum* food intake was permitted after 12:00 until the exercise test at 16:30. The results supported the findings of Clayton et al (2015), with the breakfast consumption trial eliciting a higher power output and quicker time to complete the 2000m time-trial. Interestingly, participants also reported a higher rating of perceived exertion (RPE) during the 2000m time trial (Cornford & Metcalfe, 2018).

These two studies provided an interesting proof of principle of the potential importance of breakfast for evening exercise performance. However, it is interesting to note that in both studies, despite the *ad libitum* lunchtime meal, the omission of breakfast tended to result in lower energy and carbohydrate intake across the day prior to the exercise test (Clayton et al., 2015; Cornford & Metcalfe, 2018). This raises the key question of whether the poorer exercise performance was a result of differences in meal timing *per se* or instead related to the lower carbohydrate and/or energy availability, or even the fact that participants were not blinded to the study intervention and therefore aware of what they were and were not consuming. In the most recent study on this topic, Metcalfe et al (2021) aimed to answer this question directly by "clamping" energy intake across the two trials, ensuring that there was no difference in carbohydrate and/or energy availability. Utilising a randomised crossover study design, eleven highly trained cyclists were provided with an individualised breakfast totalling 20% of their estimated daily energy requirements in a breakfast meal, and 30% of their requirements in an individualised lunchtime meal when completing the breakfast consumption trial. In the breakfast omission trial, the omission of the breakfast meal was completely compensated for at lunch, with their individualised lunchtime meal providing 50% of their estimated daily energy requirements. This meant that participants macronutrient intake was identical when it came to completing the exercise test, with the only difference being the timing of consumption. The exercise test was a 20km cycling time trial on an electronically braked cycle ergometer, performed between 5-7pm in the evening. Prior to the 20km time trial, participants were asked to complete a 10-minute steady state phase at 40% of Wmax to examine the effect of the interventions upon substrate oxidation. Carbohydrate oxidation was higher, and fat oxidation lower, in the breakfast omission trial compared to the breakfast trial, perhaps reflective of greater exogenous carbohydrate availability resulting from the larger lunchtime meal. Interestingly, despite the only difference being nutrient timing, when participants completed the breakfast omission trial, their performance in the evening 20km cycling time trial still appeared to be impaired, with mean power output ~3% lower compared to the breakfast trial (Metcalfe et al., 2021). This study provided the first evidence that the impairment of exercise performance following breakfast omission was related to meal timing *per se* rather than lower total energy and carbohydrate intake, although once more participants were not blinded to the study intervention and this must be considered when analysing the findings.

Taken together, these three studies provide some evidence that an early morning high carbohydrate meal may be important for optimising evening exercise performance. However, the mechanisms to explain these findings are unclear. Consuming a high carbohydrate breakfast is encouraged generally for athletes regardless of the exercise taking place that day, in order to increase glycogen stores, particularly liver glycogen, which can be decreased by ~40% after an overnight fast (Taylor et al., 1996). Given that glycogenolysis has been shown to be important in meeting energy demand during high sub-maximal exercise in soccer (Jacobs et al., 1982) it would seem logical to consume a high carbohydrate meal upon waking to replenish these stores.

However, a further theory bought forward by Cornford and Metcalfe (2018), and Metcalfe and colleagues (2021), is less about physiology, and more the psychological implications of omitting breakfast, even when caloric intake is clamped as in (Metcalfe et al., 2021). Both studies speculated that the results may be explained by the idea that the participants in the studies were aware that on one occasion they had not consumed a breakfast meal, which could promote a psychological expectation of reduced performance which translated to an actual reduction of performance in the exercise trials. If so, it would imply that it was indeed a psychological mechanism, rather than any physiological mechanisms occurring from the consumption/non-consumption of the breakfast meal, which caused the earlier onset of fatigue and subsequent reductions in performance markers. This explanation was also inferred in a study of the benefits of co-ingestion of protein and carbohydrate during exercise versus carbohydrate alone, with participants reporting similar levels of RPE across both exercise trials, despite completing the exercise trial at a higher intensity when co-ingesting protein and carbohydrate, inferring that participants perceived a higher exercise intensity to be no harder with the addition of protein to a carbohydrate beverage (Highton et al., 2013). This could be seen as an example of the Central Governor theory, which proposes that the subconscious brain and central nervous system regulates the body's power output by controlling motor unit recruitment to ensure that catastrophic physiological/metabolic failure never occurs, and homeostasis is continually maintained (Noakes et al., 2004). However, the central governor model of fatigue has been extensively critiqued and found to contain many flaws, with a more accurate explanation likely to fall somewhere in the middle, whereby muscle fatigue could be best described as occurring from both physiological (Peripheral fatigue) and psychological (Central fatigue) systems (Weir et al., 2006), with oscillations in activity causing differing efferent responses in what is known as the integrative governor theory, or St. Clair Gibson model (St Clair Gibson et al., 2018). Other psychological factors are also known to potentially affect exercise performance, including sensations of appetite and distractions such as music. Previous studies suggest that a placebo control can inhibit subjective appetite sensations, and subsequent exercise performance was not significantly different when compared to a breakfast meal, but both placebo and breakfast meals improved performance versus water, potentially showcasing the psychological effect of appetite on performance (Naharudin et al., 2020a). Similarly, studies have shown the effect of a form of distraction on exercise performance, most notably music. The bandwidth of human attention processing narrows during exercise and thus when a human is introduced to, or actively chooses to be distracted by something else during exercise, such as music, their perceived feelings of exertion/fatigue and other negative feelings can be seen to be reduced (Rejeski, 2016). The tempo of music has also been found to positively affect a runners perceived feeling of fatigue, with fast music helping to reduce feelings of fatigue the most (Wu et al., 2022). This showcases the variety of factors which play into the psychological effects of performance, and show that indeed, the gap between physical and mental determinants of performance are likely not too broad (Inzlicht & Marcora, 2016).

Reference:	**Group**	**Age**	**Breakfast (kcal)**	**Lunch (kcal)**	**Total caloric intake (kcal)**	**Exercise Test**	**Performance Measure**	**Key Findings**
Clayton et al. (2015)	Fasted (*n*=10) Fed (*n*=10)	22 ± 3 22 ± 3	Fed: 740 ± 47 Fasted: 0 ± 0	Fed: 1188 ± 475 Fasted: 1387 ± 434	Fed: 2708 ± 506 Fasted: 2793 ± 452	Cycling: 30 min @ 60% V̇O2 peak, 30 min maximal performance	Total Work (kJ)	Total work completed was greater in breakfast vs breakfast omission (314 ± 53 vs 300 ± 56 kJ)
Cornford & Metcalfe (2018)	Fasted (*n*=10) Fed (*n*=10)	21 ± 2 21 ± 2	Fed: 831 ± 67 Fasted: 0 ± 0	Fed: 758 ± 206 Fasted: 1236 ± 594	Fed: 1589 ± 225 Fasted: 1236 ± 594	Rowing: 2000m time trial	Power Output (W) & Time (s)	Power output was greater in breakfast vs breakfast omission, corresponding to less time taken to complete trial (465.7 ± 43.3 vs 469.2 ± 43.4 s)
Metcalfe et al. (2021)	Fasted (*n*=11) Fed (*n*=11)	25 ± 7 25 ± 7	Fed: 583 ± 54 Fasted: 0 ± 0	Fed: 874 ± 80 Fasted: 1457 ± 134	**Identical** = Complete dietary compensation	Cycling: 20km time trial	Power Output (W)	Power output 3% greater in breakfast vs breakfast omission (294 ± 56 vs 285 ± 54 W)

Table 1: Summary of results of all current studies in the research area. DER = Daily Energy Requirements

Breakfast & Afternoon/Evening Performance: Outstanding Questions and Opportunities for Further Research

It should also be noted that there are several key methodological limitations within the studies that have been conducted to date. First and foremost, none of the studies have been placebo-controlled and participants have been aware of the experimental conditions during each of the trial. Whilst it is challenging to placebo control a study of this nature, this raises the important question of whether any of the current the findings may be explained by a placebo effect. Placebo effects are known to be common in exercise performance studies; for example, a study from Ross et al., (2015) showed that the injection of a placebo improved participants performance by 1.2% in a real world, field based 3km endurance race. Similarly, but with a nutritional ergogenic aid in the form of caffeine, a study from Hurst et al., (2020) found that when middle-distance runners were given a placebo and told it was caffeine, their time to run 1,000m was like when they were given caffeine and told it was caffeine. Indeed, when runners were given caffeine, but told it was a placebo, their performance also did not improve from baseline efforts (Hurst et al., 2020). Looking at breakfast omission and performance specifically, Mears et al (2018) recently investigated the potential for placebo effects with breakfast and morning exercise performance. In a single blind trial, participants consumed either 1) a high carbohydrate semi-solid shake; 2) a taste and texture matched placebo with no nutritional content; or 3) a control trial where they only consumed water. Thirteen well-trained cyclists then completed a 10-minute steady state cycle at 60% Wmax followed by a 20km time trial on an electronically braked cycle ergometer. Despite substrate data showing a greater RER and greater carbohydrate utilisation in the carbohydrate trial, performance was not different between the placebo and carbohydrate trials during 20km time trial, but in both cases was better compared to the water only trial. Together, this provides clear evidence that the perception of breakfast (i.e., placebo) can enhance subsequent exercise performance. It is therefore important that future research in the field of breakfast and evening exercise performance employs a placebo-controlled and blinded study design.

There are several other important limitations to consider. Two out of the three studies have involved provision of the nutritional intervention outside of the lab (Cornford & Metcalfe, 2018; Metcalfe et al., 2021). This is important because, although this allows for free living conditions and can be considered more ecologically valid, there is no way to verify that participants have actually adhered to the two prescribed nutritional conditions, and this may impact upon subsequent findings. It is therefore important that future research in this area employs a fully controlled laboratory design.

It is also noteworthy that previous studies have also involved considerable time periods between the breakfast and no breakfast conditions, i.e., consuming a large, high carbohydrate breakfast or skip eating completely until 12pm/lunch. The spacing of meals can play a crucial role in meeting the nutritional goals of an athlete, with the frequency of energy intake potentially affecting metabolism and/or nutrient availability (Burke et al., 2003). A previous study found that on average team athletes had 5.2 feedings per day (Burke et al., 2003), meaning the likelihood of a four-hour period without food is relatively uncommon, and therefore it may be questioned whether such a length of time between feedings is likely to be borne out in real-world environments. To better reflect athletes eating habits, studies need to investigate smaller time differences between dietary feedings, such as delaying the high-carbohydrate breakfast rather than skipping it completely, as well as utilising a placebo as reasoned above, to match more accurately what might be seen in an athlete's preparation for competition. Therefore, the proposed study will involve manipulation of the timing only – In both conditions, a breakfast and lunch meal will be consumed, alongside a placebo to reflect the smaller periods between feedings seen in real-world athletes.

Finally, all previous studies have also involved predominantly aerobic based exercise and no study has investigated whether the effects of breakfast would also be observed during intermittent exercise that replicates the duration and demands of match play in intermittent sports, where the exercise demands vary between submaximal and maximal efforts and periods of rest. Intermittent games type exercise is most regularly played during the afternoon and evening period, and therefore is the area where the findings of this research have most potential application. As such, it seems logical to suggest that studies should focus on this mode of exercise to gain what could be considered the most valid findings for the real world.

Aims and Hypothesis of Current Research

To address the gaps in knowledge identified above, the aim of the current research study was to employ a single-blind placebo-controlled study design to determine the effect of delaying rather than completely skipping a high-carbohydrate breakfast on subsequent intermittent exercise performance in the afternoon. We hypothesised that with the utilisation of a placebo, varying the timing of feeding of a high-carbohydrate breakfast meal would have no effect on a prolonged intermittent exercise performance in the afternoon.

Method

Participants

A total of 10 participants (6 males, 4 females; age 25.6 ± 6.48 years; height 1.74 ± 0.84 m; body mass 67.9 ± 9.2 kg; BMI: 22 ± 2.5; $\dot{V}O_2max$: 48.2 ± 6.6 $ml \cdot kg^{-1} \cdot min^{-1}$; Wmax: 297 W ± 44.6 W) volunteered to take part in the study. For reasons beyond the researcher's control, including availability to take part, injuries, and dropouts, 3 participants subsequently dropped out of the study, meaning only 10 participants completed the full experimental procedures. All participants were training and playing competitive intermittent-type games sports (defined as training or playing competitively at least twice per week) and identified as habitual breakfast eaters (consuming breakfast more than five times per week). Breakfast was defined and coded as per the definition from Betts et al (2016) – "the first meal consumed within 2hrs of waking from the longest sleep in any 24hr period, that contains at least 50kcals" – participants were informed of this, and it was checked that they satisfied all conditions when consuming what they defined as a breakfast meal. All participants reported their main sport to be of an intermittent nature, and all were physically active outside of this sport by attending the gym or maintaining aerobic fitness on a regular basis, which was defined as completing at least three bouts of physical activity over 30 minutes and in addition to their main intermittent sport (activity reported as main sport by participants on the study: 1 tennis, 1 cricket, 1 netball, and 7 korfball). For female participants, study visits were arranged so as not to fall on a day in which their menstrual cycle was in the luteal phase; to negate any impact this may have on prolonged exercise performance (De Jonge, 2003).

Prior to the first visit, participants provided written consent after being provided with a full description, both in writing and verbally, of what the study protocol would involve. Following this, participants then completed a Physical Activity Readiness Questionnaire (PAR-Q) and a standardised Health History Questionnaire, including measures of height, body mass and body mass index (BMI) and blood pressure, to ensure there were no contraindications to strenuous exercise in line with ACSM pre-screening procedures (Riebe et al., 2015). If participants answered yes to any questions in the health history questionnaire, reported any injuries which may affect their ability to complete the exercise tests to the best of their ability, or presented with a resting blood pressure >140/90 mmHg, then they were not eligible to take part. The protocol was assessed and approved by the College of Engineering Ethics Committee at Swansea University and the study was conducted in accordance with the Declaration of Helsinki. As this was a placebo-controlled study and we aimed for participants to remain blinded to the experimental conditions, participants were not informed about the specific aims or hypothesis of the study and instead informed of a general aim that was intended to be vague in nature but not misleading. Specifically, participants were informed that the study was investigating "the effect of carbohydrate on afternoon exercise performance" and during the trials participants were told that each meal they were provided with 'contained energy'.

Experimental Design

This study utilised a randomised placebo controlled single blind crossover design. Participants were asked to attend the laboratory on four separate occasions (Figure 1). This included an initial visit to assess eligibility and complete a graded exercise test to determine maximal aerobic capacity ($\dot{V}O_2max$) and peak power output, followed by a second familiarisation visit to introduce participants to the design and demands of the intermittent sprint test (IST, explained below). Subsequently, participants completed two full day experimental trials (early breakfast and delayed breakfast) in a randomised and counterbalanced order. This was achieved using a randomiser website (https://www.randomizer.org/) which assigned each participant a number, 1 or 2, that denoted the two trial types.

Pre-Experimental Procedures: $\dot{V}O_2max$

During the first visit, measures of height (to the nearest 0.1m) and body mass (to the nearest 0.1kg) were taken, and then participants performed an incremental ramp exercise test to volitional exhaustion on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). The test began with a 2-minute warm up at 50 Watts (W) and then the power output was increased by 30 $W.min^{-1}$. Participants were asked to maintain a comfortable cadence (80-110 revolutions per minute (rpm)) throughout, and the test was terminated when the participant reached volitional exhaustion, which was defined as being unable to maintain a cadence of >50 rpm despite strong verbal encouragement. During the test, participants respired through a rubber face mask which was connected to an online metabolic cart that allowed breath-by-breath measurements of oxygen uptake and carbon dioxide production (Vyntus CPX, Vyaire Medical, Chicago, IL, USA). $\dot{V}O_2$max was calculated as the highest $\dot{V}O_2$ from a 15-breath rolling average during the test. Maximal heart rate and power output at the point of fatigue (Wmax) were also recorded.

Pre-Experimental Procedures: Familiarisation with IST

For the second visit, the participant returned to the laboratory to complete one half of the IST, replicating that of the main trial but with no measurements taken or nutritional interventions put in place prior to the test. As the IST contains repeated 2-minute blocks of intermittent activity (described in full below below), it was decided that one half of the IST should be sufficient to familiarise participants with the test.

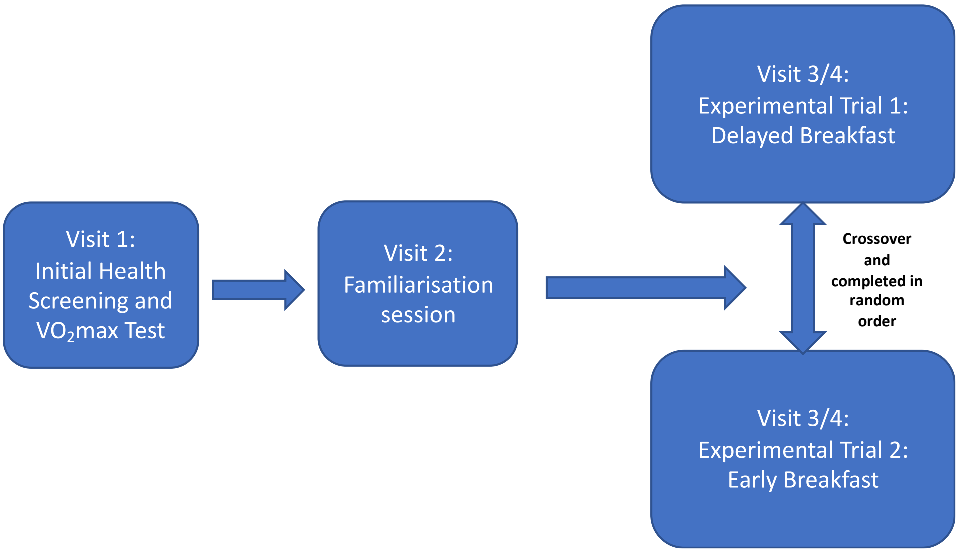

Figure 1: Diagram of the overall experimental design

Main Experimental Trials

Participants completed two full day experimental trials (early breakfast and delayed breakfast) with each trial commencing at approx. 8am and finishing at approx. 5pm. In the 24 hours prior to each main trial day, participants were asked to refrain from consuming any caffeine or alcohol. Participants were also asked not to partake in any exercise above usual physical activities of daily living, including any structured exercise training as part of their sport. Furthermore, participants were asked to record their dietary intake in an online food diary (MyFitnessPal Inc, Francisco Partners, San Francisco, CA, United States) and were subsequently asked to replicate this as accurately as possible on the day preceding the second trial.

On main trial days, participants reported fasted to the laboratory at ~7:45am, with their last meal being consumed the evening before. After resting quietly in a seated position for 10 minutes, an expired gas sample was collected to determine resting energy expenditure and substrate oxidation by indirect calorimetry (Vyntus CPX, Vyaire Medical, Chicago, IL, USA). A capillary blood sample was collected to measure blood glucose concentrations (EKF Diagnostics, Cardiff, Wales, UK) and visual analogue scales for appetite (Flint et al., 2000) were also completed. Following this, at 8am, participants were asked to consume one of two semi-solid shakes within 15 minutes. These consisted of either a high carbohydrate nutrient shake or an energy depleted but taste and texture matched 'placebo' shake (adapted from a previous paper by (Mears et al., 2018a) and described in full below). Two hours after consumption of the first shake, at 10am, participants then consumed the second (opposite) shake. In the early breakfast condition, participants consumed the high-carbohydrate shake first followed by the placebo shake two hours later, whilst in the delayed breakfast condition, participants consumed the placebo shake first followed by the high-carbohydrate shake two hours later. Two hours after the second shake was consumed, at 12pm midday, participants consumed a lunchtime meal that was in line with ISSN & UEFA guidelines on a pre-match meal (Collins et al., 2021; Kerksick et al., 2017). Subsequently, 3 hours after eating lunch, at approx. 3:30pm, participants commenced a 90-minute intermittent exercise performance test (described below).

Subjective ratings of appetite were measured every 30 minutes following the first feeding up until the IST using 100-mm visual analogue scales, and prior to the IST participants were asked to fill in a BRUMS mood scale (Terry et al., 1999). In addition, capillary blood samples were collected prior to, 30 minutes and 60 minutes after each meal, as well as prior to, at the half-time break and after the IST. Energy expenditure and substrate oxidation were also measured from a five-minute expired gas sample collected using a breath-by-breath cardiopulmonary exercise testing machine and online metabolic cart (Vyntus CPX, Vyaire Medical, Chicago, IL, USA) every hour throughout the day. In order to standardise hydration status, participants were able to drink water *ad libitum* throughout the day and during the IST during the first main trial day, and this water intake was recorded and then replicated during the second trial. A full schematic outlining the organisation of the main trial day and timing of each measurement is shown in in Figure 2.

Upon completion of the second trial an online exit questionnaire was sent to participants, to determine the success of the blinding of the experimental conditions. The exit questionnaire explained in full the reasoning behind the study and asked participants to choose in which order they thought they had consumed the semi-solid shakes on both trial days, along with a question asking for an explanation of their choice.

Meal Provision

A total of three meals were provided on each main trial day. Participants consumed two semi-solid shakes, two hours apart, which were either a placebo or contained carbohydrate. The placebo shake consisted of 6 ml·kg BM^{-1} water, 1 ml·kg BM^{-1} orange squash (Robinsons, Britvic, Hemel Hempstead, UK), 0.15 g·kg BM^{-1} Xantham gum (Doves Farm, Hungerford, UK) and 0.067 g·kg BM^{-1} artificial sweetener (MyProtein, Northwich, UK). The carbohydrate shake consisted of 6 ml·kg BM^{-1} water, 1 ml·kg BM^{-1} orange squash (Robinsons, Britvic, Hemel Hempstead, UK), 0.15 g·kg BM^{-1} Xantham gum (Doves Farm, Hungerford, UK), 0.067 g·kg BM^{-1} artificial sweetener (MyProtein, Northwich, UK) and 2 g·kg BM^{-1} maltodextrin (MyProtein, Northwich, UK).

Participants also consumed an individualised lunchtime meal consisting of Tomato & Basil Pasta (Tesco, Welwyn Garden City, UK), a cereal bar (Nature Valley, General Mills, United States of America), a yoghurt and muesli blend (Activia, Danone Groupe, France) and orange juice (Tesco, Welwyn Garden City, UK). The amount of each food consumed was based on the participants estimated resting energy expenditure, derived from the Harris and Benedict equation, revised by Mifflin et al (1990), and then multiplied by a physical activity level of 1.75. It was designed to provide approximately 30% of the participants daily energy requirements, and to meet the macronutrient guidelines from the ISSN and UEFA for a pre-match meal (Collins et al., 2021; Kerksick et al., 2017). Where necessary, providing 30% of a participants daily energy requirements was priortisied. The composition of the lunch meal was identical in both trials. Across the first trial, participants hydration was monitored using a marked water bottle, with consumption across the day recorded and replicated in the second trial.

Appetite Assessment

Visual analogue scales (100 mm) to measure different dimensions of appetite, been shown to be reliable for appetite research (Flint et al., 2000), were completed every 30 minutes throughout the main trial days. Questions were related to perceived hunger, fullness, prospective food consumption and desire to eat, with an overall appetite rating calculated using the formula: Desire to Eat + Hunger + (100 – Fullness) + Prospective Consumption.

Capillary Blood Sample Collection and Analysis

All blood samples were taken from the participants fingertips using an aseptic technique and collected into a 20 µl capillary tube, before being placed into an Eppendorf containing 1000 µl of haemolysing solution (EKF Diagnostics, Cardiff, Wales, UK). After collection, samples were analysed for blood glucose and blood lactate concentrations (Biosen C-Line, EKF Diagnostics, Cardiff, Wales, UK). A total of 12 capillary blood samples were collected per trial.

Indirect Calorimetry

Expired gas samples were analysed for expired Oxygen (O_2) and Carbon Dioxide (CO_2) concentrations as well as respiratory exchange ratios. The final three minutes of each five-minute sample were used to determine substrate (carbohydrate and fat) oxidation and energy expenditure using the stoichiometric equations published by (Frayn, 1983). Immediately prior to the first sample and then periodically throughout the day, the metabolic cart was calibrated using gases of known concentration and a digital volume transducer as per the manufacturer's instructions. The Vyntus CPX has been reported to provide highly accurate and reproducible measurements with regard to both cardiopulmonary exercise testing (CPET) and resting energy expenditure (Souren et al., 2021).

The Intermittent Sprint Test (IST)

Approximately 3 hours after the lunchtime meal, the participant completed the intermittent sprint test (IST). The IST was performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), with a cycling intermittent sprint protocol shown to provide good reliability in relation to intermittent sprint performance, with peak power output being the most reliable variable, displaying a 2.9% typical error of measurement between trials (Hayes et al., 2013). Prior to the IST beginning, participants completed a standardised 4-minute warm-up, consisting of 2 minutes cycling at 50 W, followed by 1 minute at 50% of participants Wmax, followed by 50 W for 1 minute prior to the full IST commencing.

The test was designed to replicate the movement pattern of intermittent games-type exercise and was adapted from a previously published paper (Bishop & Claudius, 2005). The IST consisted of two halves of approximately 37 minutes. Each half involved repeated ~2-min blocks that comprised of a 100 second cycle at 35% of the participants Wmax, which was shown by Bishop & Claudius (2005) to represent the intensity at which intermittent games players spend the majority of their time at, a 15 second period of unloaded pedalling, followed by a 6 s all-out sprint with a torque factor of 0.7nm/kg for males and 0.6nm/kg for females. Participants were given a 3 s window to increase their cadence prior to the sprint commencing and were instructed to sprint maximally and maintain their highest possible cadence throughout each sprint.

Two repeated sprint bouts were also completed in each half, after the 8th and 16th sprint. These consisted of four, 4 s sprints, interspersed with 15 s of active recovery (cycling at 35% Wmax). These repeated sprint bouts replaced the 9th and 17th blocks, meaning there was a total of twenty, ~2-minute blocks per half of the IST (**Figure 3)**. These were added to ensure the test matched the movement pattern of intermittent exercise as accurately as possible, using evidence from a time motion analysis of an international field hockey game, a sport which displays high correlation with football when comparing intermittent exercise profiles. This research identified that players complete an average of 2 repeated sprint bouts per game (Spencer et al., 2004), whilst a similar study of elite footballers found an average of 2.2 repeated sprint bouts per match, with an average of 15.9 s recovery that tended to be active in nature (Carling et al., 2012), thus four second sprints were chosen with 15 s of recovery to best replicate this in the IST.

Participants were given a 10-minute break between the two halves of the test. During this time participants were allowed to step of the bike and either rest in a seated position or walk around the lab. They were also provided with an isotonic energy gel containing 22g of carbohydrate (Orange Flavour, Science in Sport, London, UK), to simulate typical nutritional practices in team sports (Abreu et al., 2021). In addition, participants were allowed to drink cold water from a pressurised dispenser *ad libitum* throughout the entirety of the IST during the first trial, and the amount of water consumed was recorded, and subsequently replicated in the second trial.

Power output was recorded continuously during the test (Lode Ergometry Manager, Lode, Groningen, The Netherlands), and from this peak power output, average power output, minimum end power output and fatigue index from each sprint were calculated. This was analysed using the raw data from each test. Peak power output was taken as the highest power output during each sprint, with average power calculated by taking the average power output from the entirety of each 6-second sprint / 4-second repeated sprint. Minimum end power was taken as the lowest power output recorded after peak power during each 6-second sprint / 4-second repeated sprint. Finally, fatigue index was calculated by taking the minimum power away from the maximal power and then dividing by the total time for the sprint. During the test, participants were also asked to give their rating of perceived exertion (RPE) after each sprint. Participants heart rate after each sprint was also recorded, using a heart rate monitor and watch (Polar Electro Oy, Kempele, Finland).

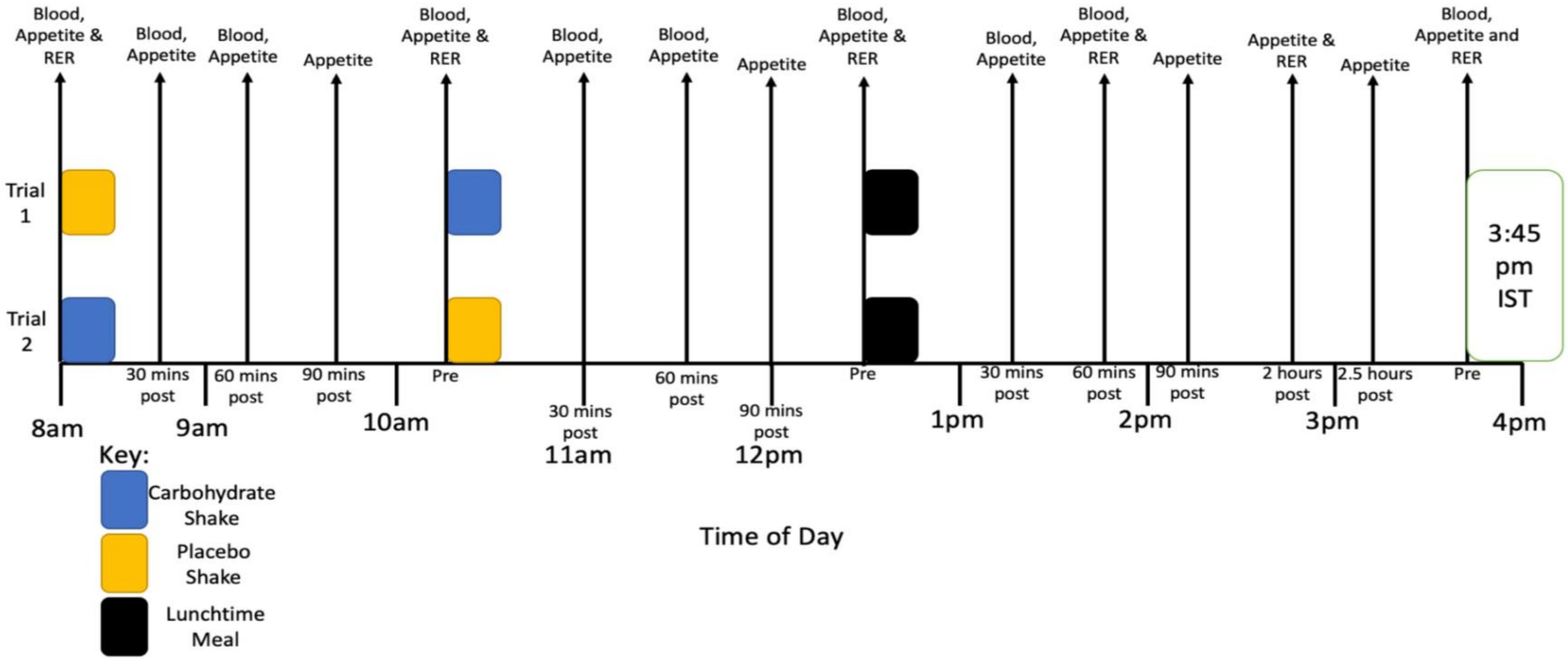

Figure 2: Schematic representation of the main trial days for the study. Participants entered the lab approximately 15 minutes before consuming the first shake and finished the day around 5:30pm after completion of the IST.

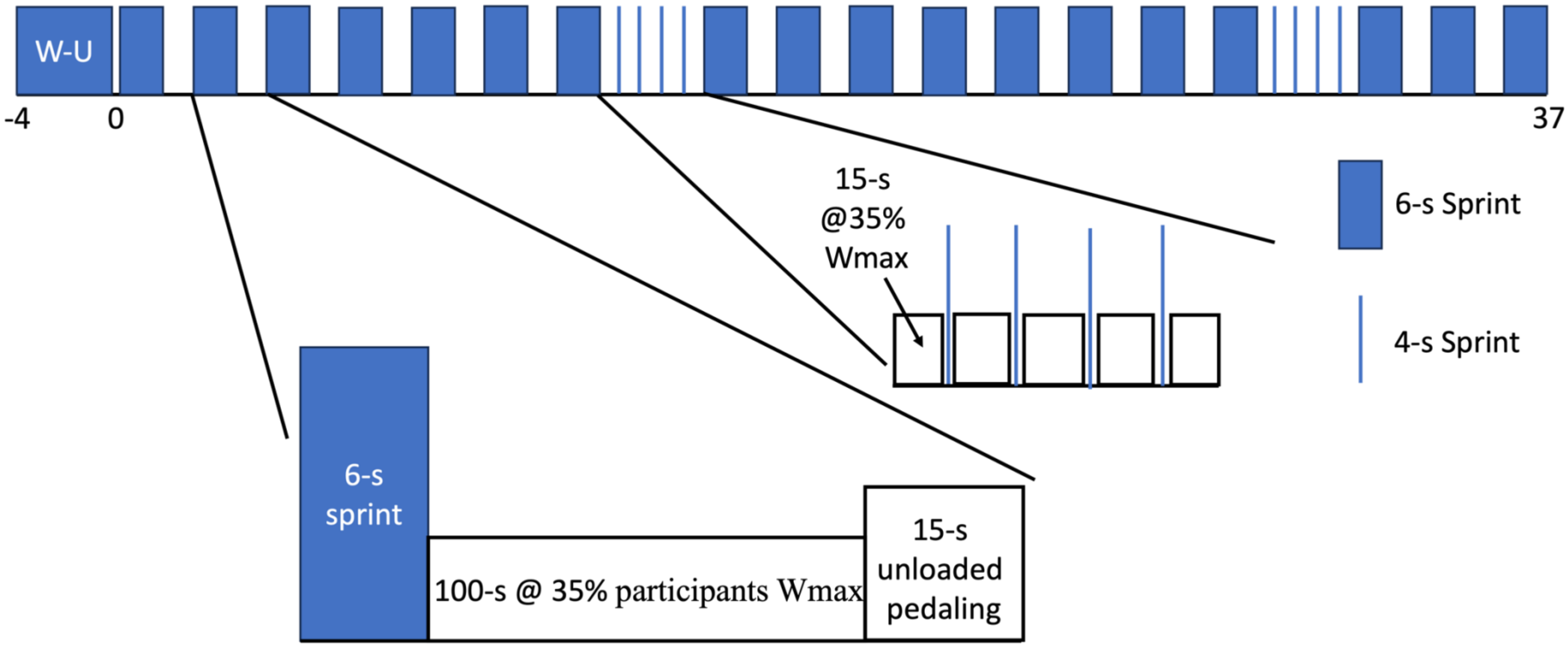

Figure 3: Schematic representation of the first half of the Intermittent Sprint Test (IST). Each block consisted of a 6-s all out sprint, 100-s at 35% of a participants Wmax followed by 15-s unloaded pedalling. The two repeated sprint blocks consisted of 4x4-s sprints, interspersed with 15-s active recovery @ 35% of participants Wmax. W-U; warm-up, timeline in minutes.

Statistical Analysis

Based on two previous studies (Cornford and Metcalfe, 2018; Metcalfe 2020), the effect size (dz, mean difference / SD of the difference) for the effect of breakfast omission on evening performance parameters is between 0.78 and 0.94. Taking the more conservative estimate (0.78), using G*Power, and an alpha 0.05 for a two-sided test, we calculated that n=15 participants will provide 80% power to detect a similar effect size in this study. Data from the study was analysed using GraphPad Prism 10 for MacOS (Version 9.1.0, San Diego, CA, USA), with all data first being checked for normality of distribution using the Shapiro-Wilks test. Data containing only one factor, EARLY vs DELAY (e.g., mood prior to the IST), were analysed using a paired t-test if normally distributed, or a Wilcoxon signed rank test if non-normally distributed. Data containing an additional factor of time (All performance measures, appetite ratings, substrate oxidation, blood glucose and RER through the day) were analysed using a two-way repeated measures ANOVA, for both normal and non-normal distribution. Effect sizes were also calculated for all data containing an additional factor of time using Cohens D calculation. If a significant interaction effect was found, post-hoc comparisons between the same time point on the two trials were made where appropriate using the Fishers Least Significant Difference test. For glucose, appetite and substrate data, area under the curve analysis was performed, for both total area under the curve, and incremental area under the curve. Area under the curve analysis was performed using the time series response analyser (Narang et al., 2020) to reduce the chance of human error. Statistical significance for all analysis was accepted at $p<0.05$, and all data are presented as mean and standard error of mean (unless otherwise explicitly stated).

Results

Blood Glucose Responses

The blood glucose response during the two trials is shown in **Figure 1**. There was a main effect of time and a condition x time interaction effect between the two trials for blood glucose (both $p < 0.001$) indicating a different pattern of response for blood glucose between the two trials, but no main effect of condition between EARLY and DELAY ($p < 0.058$). Blood glucose levels were similar at baseline, but following the first shake, there was an increase in blood glucose in the EARLY but not the DELAY breakfast trial, such that blood glucose was higher in the EARLY breakfast trial for the following two hours (all $p<0.05$, **Figure 1**). Following the second shake, there was an increase in blood glucose in DELAY, but blood glucose continued to fall in EARLY (**Figure 1**). There were no significant differences in blood glucose concentrations between the EARLY and DELAY trials at any time point following shake 2 (**Figure 1**).

Incremental (iAUC) and total (tAUC) area under the curve analysis was also completed for blood glucose (**Table 2**). For iAUC, paired t-tests between conditions at each timepoint showed a significant difference between EARLY and DELAY between 0-120 minutes ($p < 0.0002$) and 120-240 minutes ($p < 0.0001$), but no significant differences between 240-420 minutes, or across the full period. For tAUC, significant differences were seen across the two conditions between 0-120 minutes ($p < 0.0003$) and over the full period (0-420 minutes; $p < 0.003$) but not between 120-240 minutes, or 240-420 minutes. Comparisons were also made between the 0-120 minute and 120-240 minute timepoints in EARLY vs DELAY; For iAUC there were no significant differences between timepoints. For tAUC there was a significant difference between EARLY 0-120 minutes vs DELAY 120-420 minutes (544.03 mmol/L/120 mins *vs* 633.13 mmol/L/120 mins; $p < 0.0004$) and EARLY 120-420 minutes vs DELAY 0-120 minutes (662.3 mmol/L/120 mins *vs* 739.46 mmol/L/120 mins; $p < 0.02$).

	0 – 120 mins	**120 – 240 mins**	**240 – 420 mins**	**0 – 420 mins**
iAUC (mmol/L/420 mins)				
Early Breakfast	161.12 ± 88.07	5.46 ± 10.88	49.74 ± 73.22	294.41 ± 140.59
Delayed Breakfast	5.64 ± 9.22	130.52 ± 59.62	10.26 ± 17.33	186.09 ± 122.36
tAUC (mmol/L/420 mins)				
Early Breakfast	730.16 ± 97.32	626.93 ± 44.87	924.8 ± 80.26	2268.76 ± 168.39
Delayed Breakfast	537.93 ± 63.29	653.64 ± 49.87	915.8 ± 79.86	2104.87 ± 172.69

Table 2: Area under the curve analysis for each defined time period in the EARLY and DELAY trials, for both Incremental (iAUC) and Total (tAUC) area under the curve. Data is presented as mean ± SD.

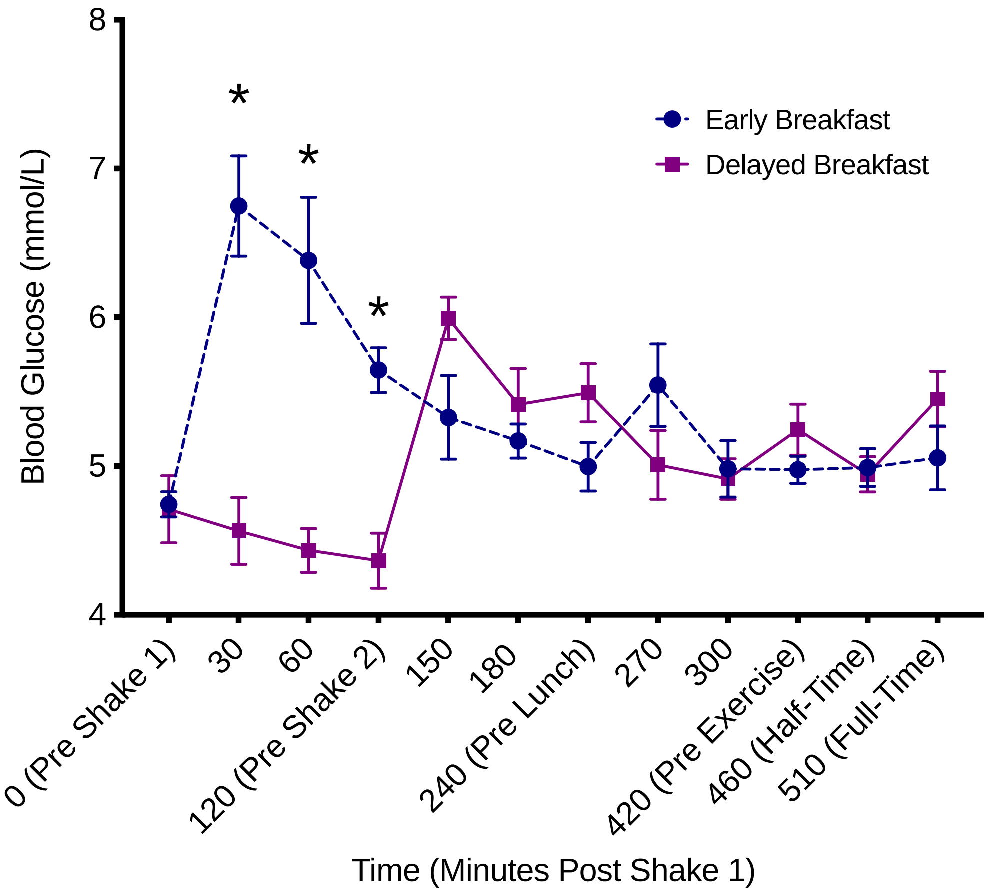

Figure 1: Blood glucose concentrations during the EARLY and DELAY trials. Data is presented as mean ± SEM for greater visual clarity. * Indicates a significant difference between the two trials (p <0.05).

Substrate Oxidation

Due to technical difficulties with equipment at one timepoint during a trial, substrate oxidation data from only nine participants was available for analysis. There was a significant main effect of time and a time x condition interaction effect for participants respiratory exchange ratio (both p <0.0001), but no main effect of condition (p < 0.25). There was an increase in RER following shake one in EARLY but not the DELAY trial and RER was higher in the EARLY trial for the following two hours (both p<0.05). Following shake two, there was an increase in RER in the DELAY breakfast trial, whereas RER remained similar in the EARLY breakfast trial.

Both carbohydrate and fat oxidation showed a main effect of time (both p<0.0001) and a time x condition interaction effect (both p<0.001, **Figure 2A and 2B**), but no main effect of condition between EARLY and DELAY (carbohydrate oxidation, p < 0.54; fat oxidation, p < 0.33). *Post-hoc* analysis revealed higher rates of carbohydrate oxidation at 60- and 120-minutes following shake one in EARLY compared to the DELAY breakfast trial (both p<0.05, **Figure 2A**), whilst rates of fat oxidation were significantly lower in the EARLY breakfast trial at 120 minutes only (p<0.05, **Figure 2B**). There were no significant differences in rates of carbohydrate and fat oxidation following shake two and for the rest of the afternoon (**Figure 2A and 2B**).

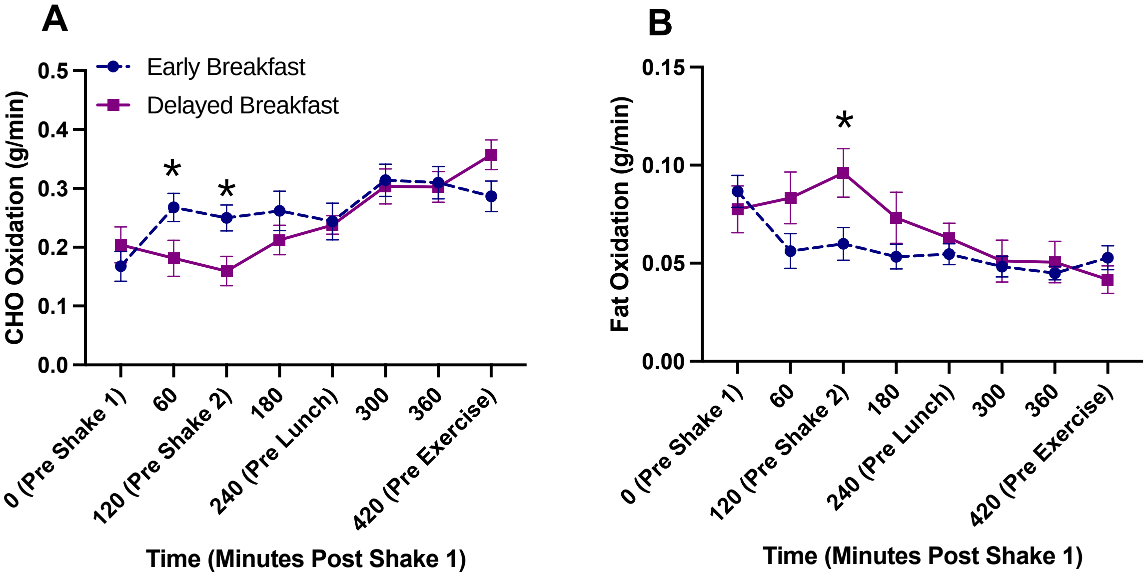

Figure 2: Rates of carbohydrate and fat oxidation during the day in the EARLY and DELAY conditions (n=9). Data is presented as mean ± SEM for greater visual clarity. * Represents a significant difference between the two trials ($p<0.05$).

Incremental (iAUC) and total (tAUC) area under the curve analysis was also completed for both carbohydrate **(Table 3)** and fat oxidation (**Table 4**).

For carbohydrate oxidation, iAUC values between condition showed a significant difference at 0-120 minutes ($p < 0.02$), but not at 120-240 minutes, 240-420 minutes, or the full period (0-420 minutes). For tAUC, values between conditions at each timepoint showed a significant difference between EARLY and DELAY at 0-120 minutes ($p < 0.02$) and 120-240 minutes ($p < 0.04$), but there was not a significant difference between 240-420 minutes, or across the full 420 minutes. Comparisons across timepoints were also made. For iAUC, no significant differences were found at any comparison point. For tAUC, when comparing EARLY 0-120 minutes vs DELAY 120-240 minutes, a significant difference was found (21.78g/120 mins *vs* 32.87g/120 mins; $p < 0.01$), however when comparing EARLY 120-240 minutes vs DELAY 0-120 minutes, there was no significant difference.

	0 – 120 mins	**120 – 240 mins**	**240 – 420 mins**	**0 – 420 mins**
		iAUC (g/420 mins)		
Early Breakfast	8.82 ± 4.34	2.95 ± 2.30	9.72 ± 7.98	48.55 ± 32.52
Delayed Breakfast	2.20 ± 5.14	5.87 ± 3.28	12.02 ± 7.59	27.24 ± 31.63
		tAUC (g/420 mins)		
Early Breakfast	28.91 ± 6.05	24.66 ± 6.79	54.21 ± 11.85	100.65 ± 22.93
Delayed Breakfast	21.78 ± 8.95	32.87 ± 10.20	56.94 ± 14.57	118.74 ± 28.99

Table 3: Area under the curve analysis for CHO oxidation, for each defined time period in the EARLY and DELAY trials, for both Incremental (iAUC) and Total (tAUC) area under the curve. Data is presented as mean ± SD

For fat oxidation **(Table 4)**, iAUC analysis showed a significant difference between EARLY and DELAY at 0-120 minutes ($p < 0.01$) but at no other timepoint. For tAUC, there was no significant difference at 0-120 minutes, but there was at 120-420 minutes ($p < 0.03$). There were no further significant differences at any later timepoints, or the full day. When comparisons were made across timepoints, iAUC showed a significant difference in EARLY 0-120 minutes *vs* DELAY 120-240 minutes (1.68g/120 mins *vs* 0.11g/120 mins; $p < 0.03$) but not across any other timepoint comparison. For tAUC, there was also a significant difference in EARLY 0-120 minutes *vs* DELAY 120-240 minutes (10.21g/120 mins *vs* 6.76g/120 mins; $p < 0.006$) but not between EARLY 120-240 minutes vs DELAY 0-120 minutes.

	0 – 120 mins	**120 – 240 mins**	**240 – 420 mins**	**0 – 420 mins**
iAUC (g/420 mins)				
Early Breakfast	0.009 ± 0.03	0.11 ± 0.1	0.38 ± 0.53	0.04 ± 0.09
Delayed Breakfast	1.68 ± 1.73	0.006 ± 0.02	0.39 ± 0.76	4.89 ± 7.28
tAUC (g/420 mins)				
Early Breakfast	7.94 ± 2.78	6.76 ± 2.09	9 ± 1.92	23.7 ± 6.27
Delayed Breakfast	10.21 ± 3.98	9.16 ± 3.97	9.23 ± 4.63	28.6 ± 11.62

Table 4: Area under the curve analysis for Fat oxidation, for each defined time period in the EARLY and DELAY trials, for both Incremental (iAUC) and Total (tAUC) area under the curve. Data is presented as mean ± SD

Subjective Appetite Responses

Each of the four components of appetite (hunger, desire to eat, fullness, prospective food consumption), as well as the composite appetite score, showed a main effect of time (*p*<0.0001 for all) however there was no main effect of condition. The pattern of change in appetite ratings over time was as would be expected following the consumption of each meal, i.e., a clear suppression of appetite following each provided meal followed by a steady rise in appetite in the following hours (**Figure 3**). Crucially, there were no significant time x condition interaction effects for any appetite measure (all p>0.05) indicating that the pattern of change over time was similar in the EARLY and DELAY conditions (**Figure 3**).

Total (tAUC) area under the curve analysis was also completed for participants composite appetite score (**Table 5**). Paired t-tests between tAUC values between conditions at each timepoint (0-120 minutes, 120-240 minutes, 240-420 minutes and 0-420 minutes) showed no significant differences. Comparisons were also made between the 0-120 minute and 120–240-minute timepoints in EARLY vs DELAY; there were also no significant differences seen.

	0 – 120 mins	**120 – 240 mins**	**240 – 420 mins**	**0 – 420 mins**
tAUC (mm/420 mins)				
Early Breakfast	6531.41 ± 1322.27	6541.88 ± 1733.77	6791.44 ± 2908.66	19,684.5 ± 4722.21
Delayed Breakfast	6139.55 ± 1624.17	5474.63 ± 2096.96	7386.56 ± 2418.36	19,000.5 ± 5364.18

Table 5: Area under the curve analysis for each defined time period of appetite measurements in the EARLY and DELAY trials, for both Incremental (iAUC) and Total (tAUC) area under the curve. Data is presented as mean ± SD.

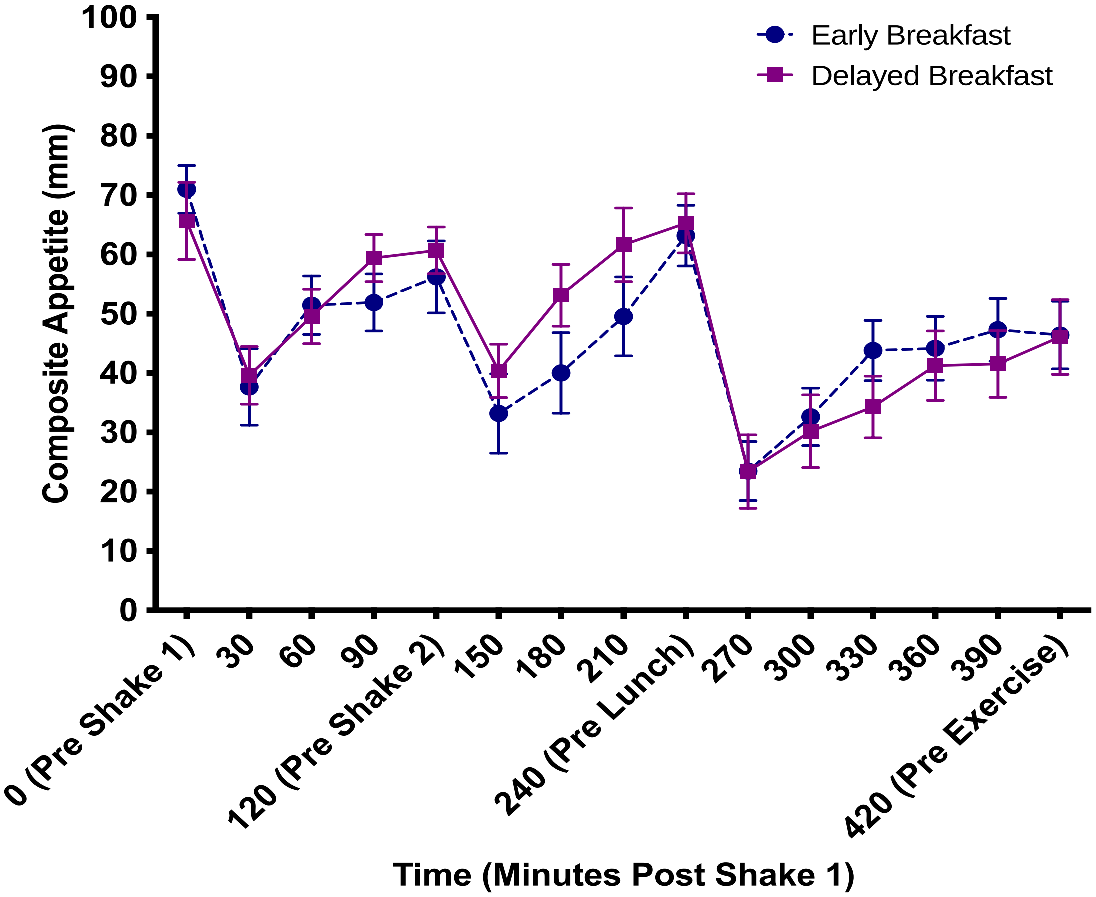

Figure 3: Composite Appetite (3E) scores throughout the day in the EARLY and DELAY trials. Data is presented as mean ± SEM for greater visual clarity.

Intermittent Exercise Performance

Peak Power During Intermittent Sprints

Peak power during the 6-s sprints in the first and second halves of the intermittent exercise test is shown in **Figure 4A** and **Figure 4C**, respectively. There was a significant main effect of time (both halves $p<0.0001$); however, there was no main effect of condition and no time x condition interaction effect. A simple paired t-test of the mean of the peak power from all sprints in each half also showed no significant difference between the EARLY and DELAY conditions and Cohens D effect sizes were all negligible (first half: $p=0.74$, d=0.01; second half: $p=0.60$, d=0.02; **Figure 4B and 4D**).

Peak Power During Repeated Sprints

Peak power during the two blocks of repeated sprints that were embedded during the first half and the second half of the intermittent exercise test are shown in **Figure 5A** (First half) and **Figure 5C** (Second half) respectively. There was a significant effect of time in both halves (*p*<0.0001) indicative of the progressive decrease in peak power as the number of sprints progressed (**Figure 5**). However, there was no significant effect of condition and no time x condition interaction effect suggesting no effect of breakfast timing. A simple paired t-test of the mean of the peak power from all repeated sprints in each half also showed no significant difference between the EARLY and DELAY conditions and Cohens D effect sizes were all negligible (first half: *p*=0.22, d=0.08; second half: *p*=0.35, d=0.06; **Figure 5B and 5D**)

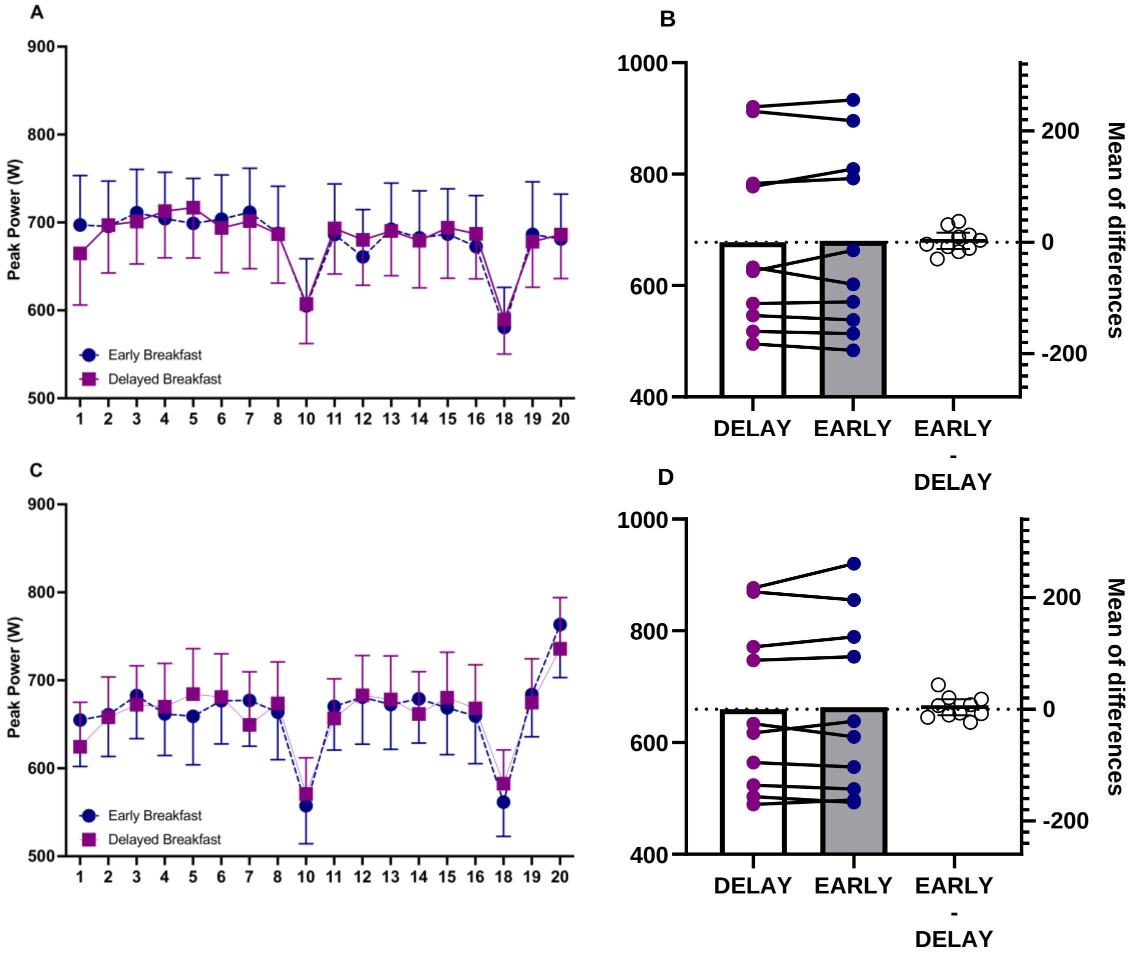

Figure 4: Peak power during each intermittent sprint in the first half (4A) and the second half (4C), alongside the peak power averaged across all sprints in the first

half (4B) and the second half (4D). Data in 5A and 5C is presented as mean ± SEM. In 4B and 4D, data on the left-hand axis is presented as the mean with individual data points overlaid, whilst the right-hand axis of 4B and 4D represents the mean difference between conditions ± 95% CI, with clear dots representing individual participant difference scores.

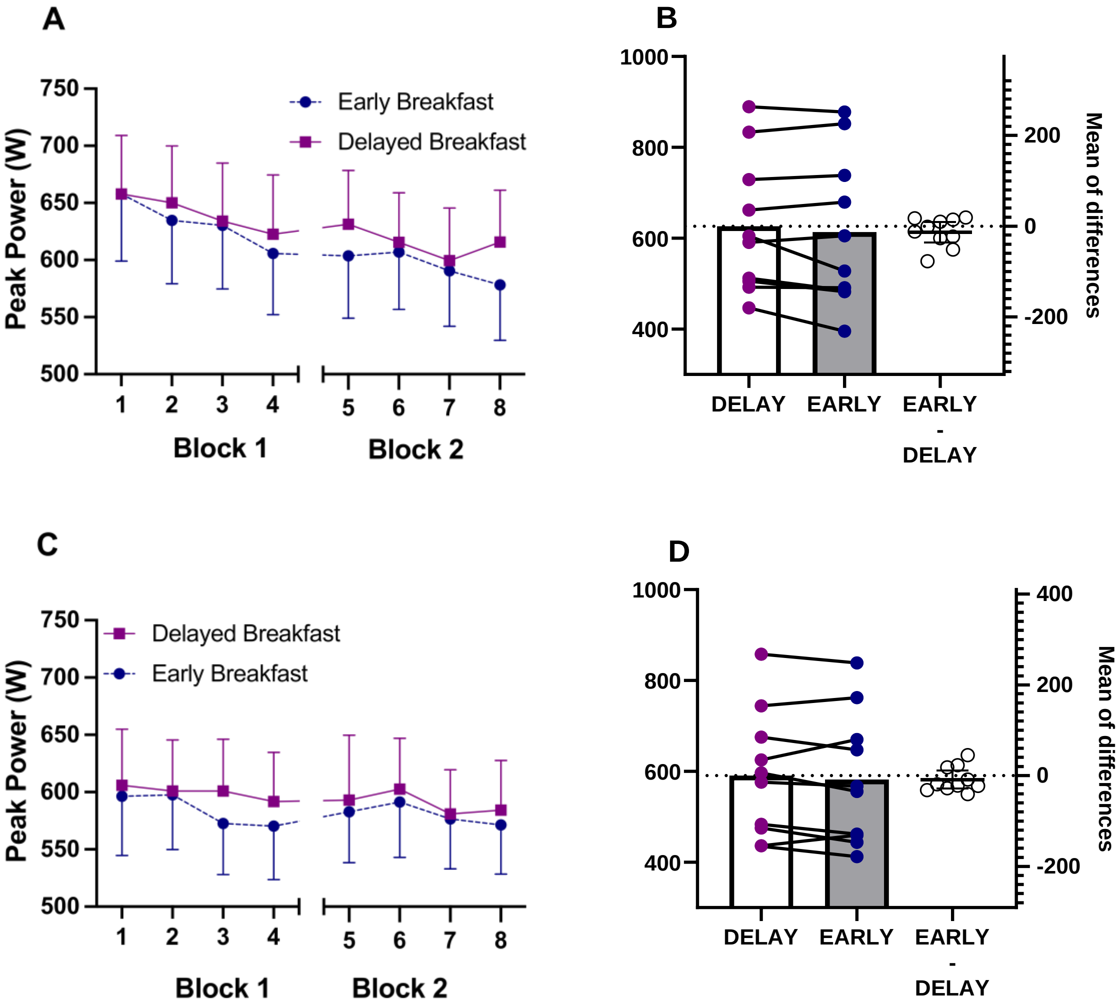

Figure 5: Peak power during each repeated sprint in the first half (5A) and the second half (5C), alongside the peak power averaged across all repeated sprints in the first half (5B) and the second half (5D). Data in 5A and 5C is presented as mean ± SEM. Data on the left-hand axis of 5B and 5D is presented as the mean with individual data points overlaid, whilst the right-hand axis represents the mean difference between conditions ± 95% CI, with clear dots representing individual participant difference scores.

Average Power During Intermittent Sprints

There was a significant main effect of time for average power during the intermittent sprints in both the first and second halves (**Figure 6A & Figure 6C;** both halves main effect of time p<0.0001). However, there was no main effect of condition and no condition x time interaction effect. A simple paired t-test of the mean of the average power from all sprints in each half also showed no significant difference between the EARLY and DELAY conditions and Cohens D effect sizes were all negligible (first half: p=0.84, d=0.01; second half: p=0.55, d=0.02; **Figure 6B and 6D**).

Average Power During Repeated Sprints

Average power during the two blocks of repeated sprints that were embedded during the first half and the second half of the intermittent exercise test are shown in **Figure 7A** (First half) and **Figure 7C** (second half) respectively. There was a significant effect of time in both halves (p<0.0001) indicative of a continual decrease in average power as the repeated sprints progressed through the test (**Figure 7A and Figure 7C**). However, there was no significant effect of condition and no time x condition interaction effect, suggesting there was no impact of breakfast timing. A simple paired t-test of the mean of the average power from all repeated sprints in each half also showed no significant difference between the EARLY and DELAY conditions and Cohens D effect sizes were all negligible (first half: p=0.27, d=0.08; second half: p=0.53, d=0.04; **Figure 7B and 7D**).

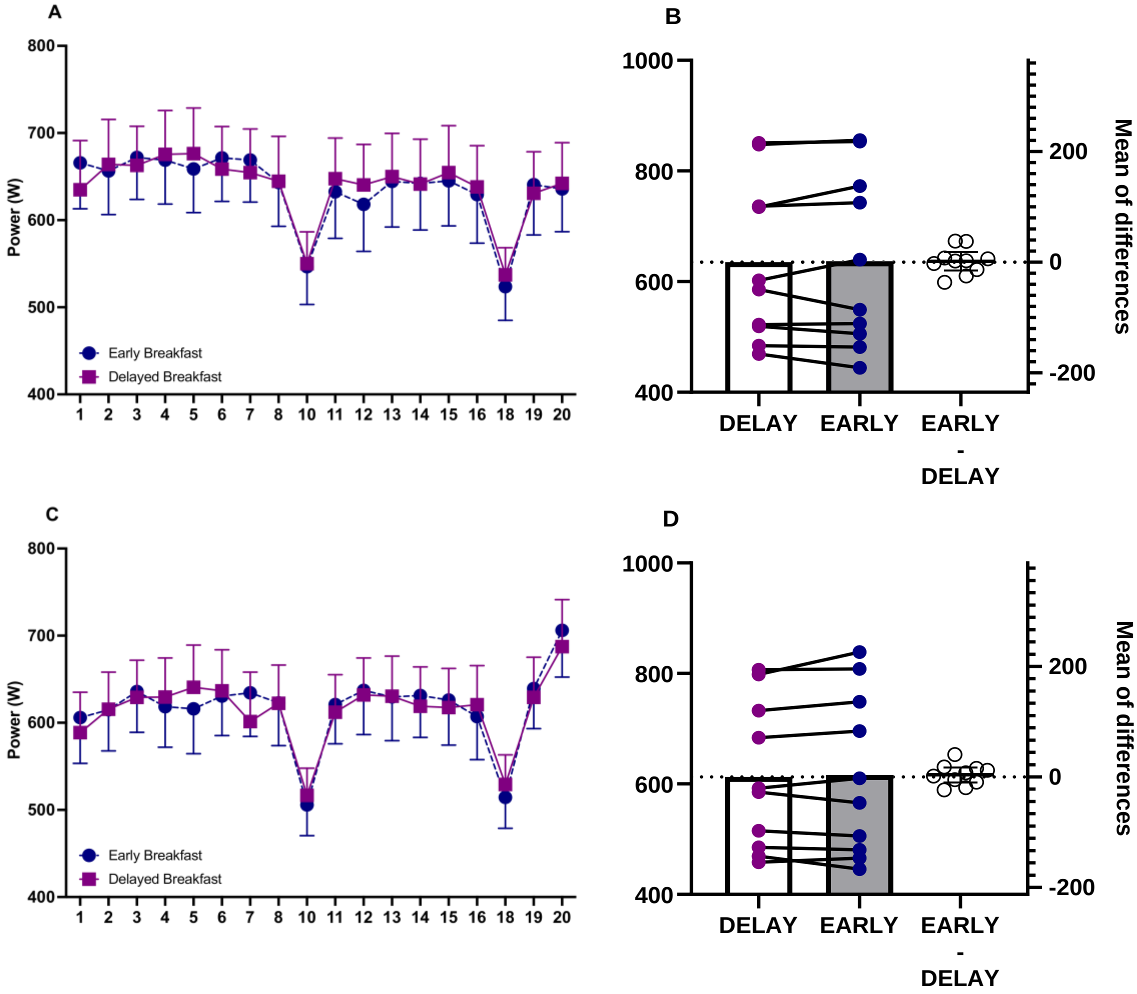

Figure 6: Average power during each intermittent sprint in the first half (6A) and the second half (6C), alongside the average power averaged across all sprints in the first half (7B) and the second half (6D). Data in 7A and 7C is presented as mean ± SEM Data on the left-hand axis of 6B and 6D is presented as the mean with individual data points overlaid, whilst the right-hand axis represents the mean difference between conditions ± 95% CI, with clear dots representing individual participant difference scores.

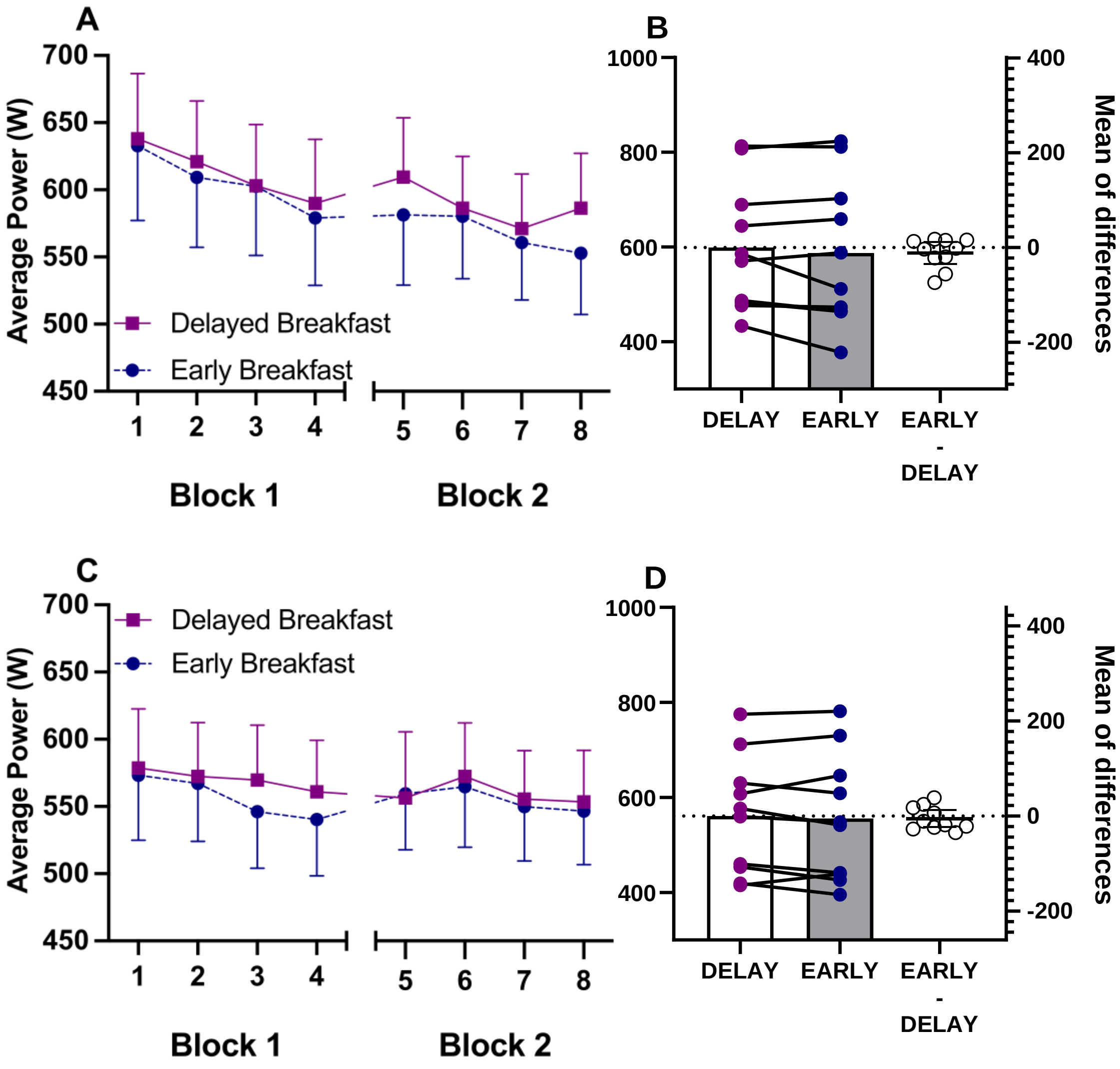

Figure 7: Average power during each repeated sprint in the first half (7A) and the second half (7C), alongside the average power averaged across all repeated sprints in the first half (7B) and the second half (7D). Data in 7A and 7C is presented as mean ± SEM. Data on the left-hand axis of 7B and 7D is presented as the mean with individual data points overlaid, whilst the right-hand axis represents the mean difference between conditions ± 95% CI, with clear dots representing individual participant difference scores.

Minimum Power During Intermittent Sprints

The minimum power output during intermittent sprints in the first half **(Figure 8A)** and second half **(Figure 8C)** showed a significant main effect of time across both halves (*p*<0.0001); however, there were not a significant main effect of condition and no condition x time interaction effect, displaying the lack of effect breakfast timing had on performance. A simple paired t-test of the mean of the minimum power from all sprints in each half also showed no significant difference between the EARLY and DELAY conditions and Cohens D effect sizes were all negligible (first half: *p*=0.77, d=0.02; second half: *p*=0.69, d=0.02; **Figure 8B and 8D**).

Minimum Power During Repeated Sprints

Minimum power during the two blocks of repeated sprints that were embedded during the first half and the second half of the intermittent exercise test are shown in **Figure 9A** (First half) and **Figure 9C** (Second half) respectively. There was a significant effect of time in both halves (*p*<0.0001); however, no significant effect of condition and no time x condition interaction effect was seen. A simple paired t-test of the mean of the minimum power from all repeated sprints in each half also showed no significant difference between the EARLY and DELAY conditions and Cohens D effect sizes were all negligible (first half: *p*=0.33, d=0.08; second half: *p*=0.49, d=0.04; **Figure 9B and 9D**)

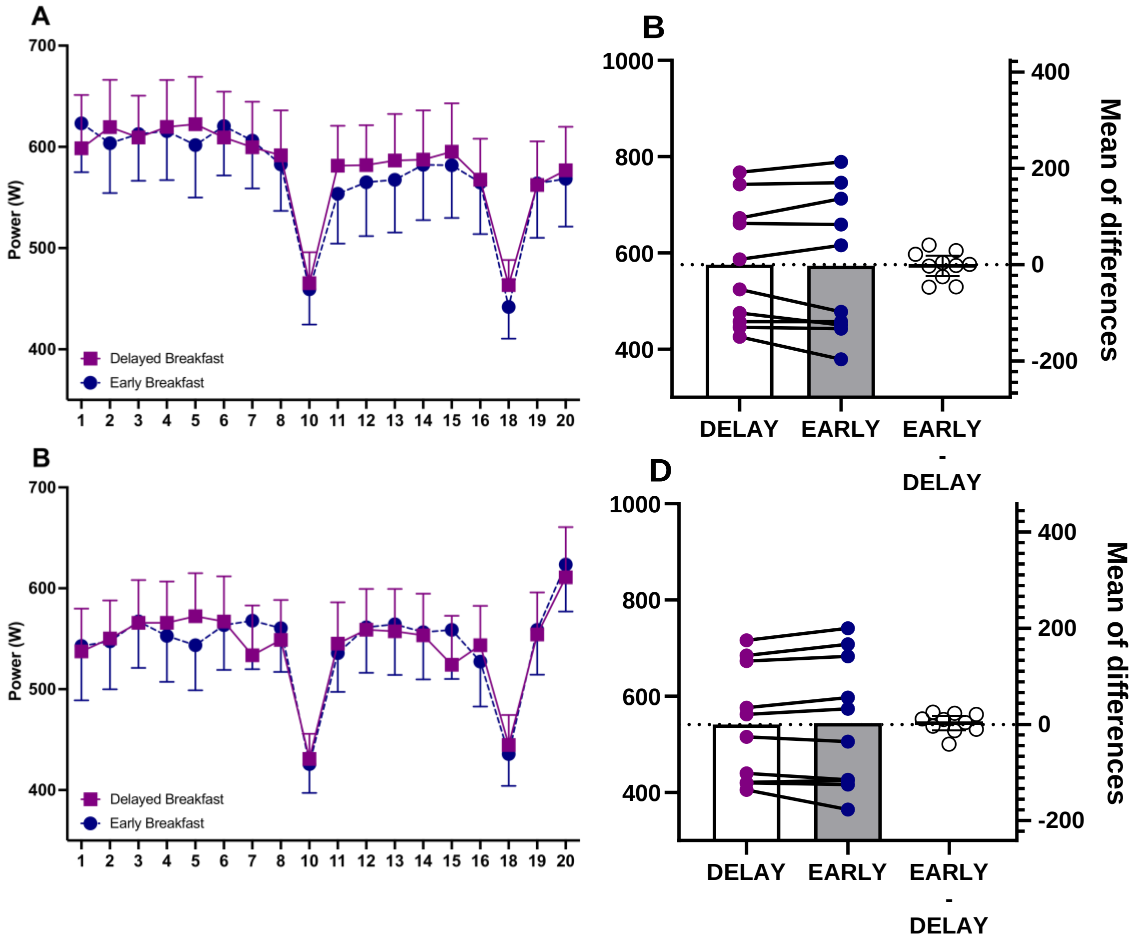

Figure 8: Minimum power during each intermittent sprint in the first half (8A) and the second half (8C), alongside the minimum power averaged across all sprints in the first half (8B) and the second half (8D). Data in 8A and 8C is presented as mean ± SEM. Data on the left-hand axis of 8B and 8D is presented as the mean with individual data points overlaid, whilst the right-hand axis represents the mean difference between conditions ± 95% CI, with clear dots representing individual participant difference scores.

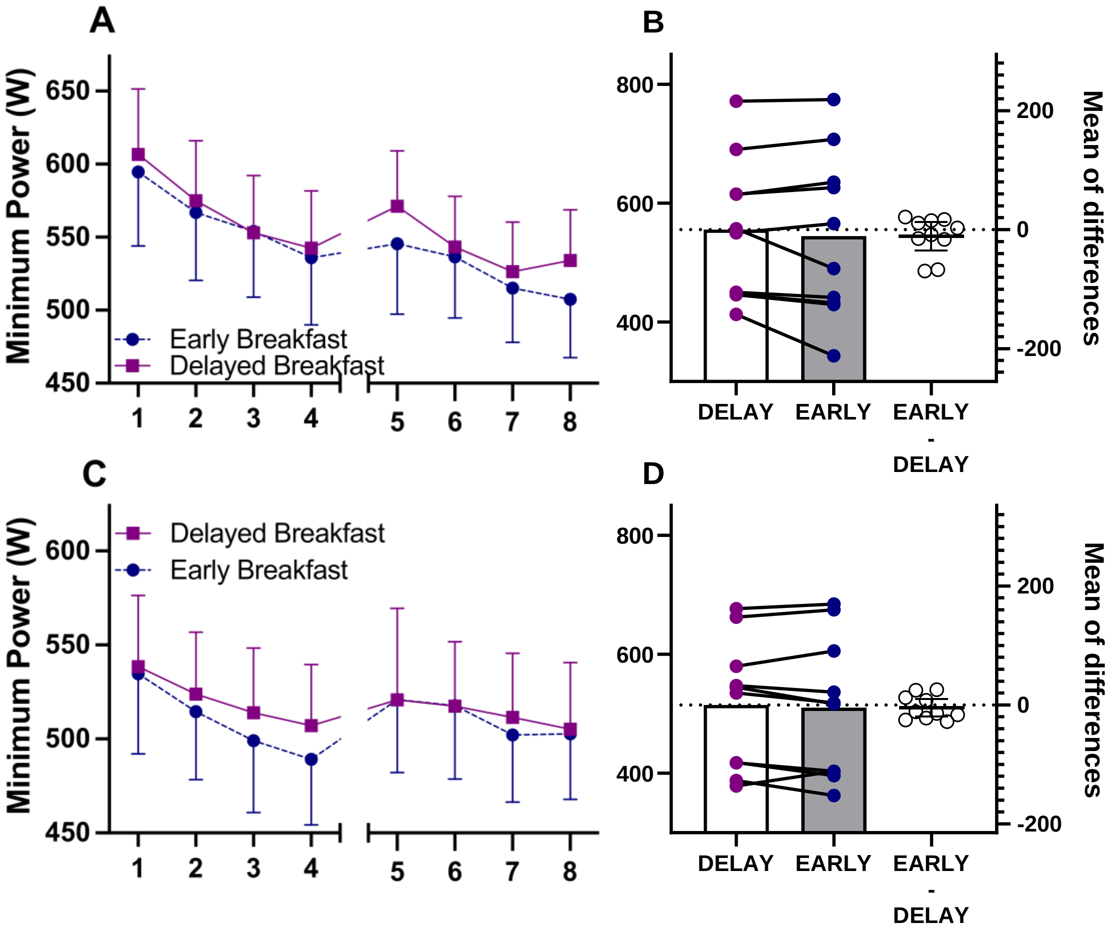

Figure 9: Minimum power achieved from each repeated sprint in the first half (9A) and the second half (9C), alongside the minimum power averaged across all repeated sprints in the first half (9B) and the second half (9D). Data in 9A and 9C is presented as mean ± SEM. Data on the left-hand axis of 9B and 9D is presented as the mean with individual data points overlaid, whilst the right-hand axis represents the mean difference between conditions ± 95% CI, with clear dots representing individual participant difference scores.

Fatigue Index During Intermittent Sprints

The fatigue index during intermittent sprints in the first half **(Figure 10A)** and second half **(Figure 10C)** showed a significant main effect of time in the first half (*p*<0.002) but not the second half (*p*=0.20). There were no significant main effects of condition and no time x condition interaction effect in either the halves of the test, and a simple paired t-test of the mean of the fatigue index from all sprints in each half also showed no significant difference between the EARLY and DELAY conditions, with Cohens D effect sizes all negligible (first half: *p*=0.43, d=0.12; second half: *p*=0.93, d=0.01; **Figure 10B and 10D**).

Fatigue Index During Repeated Sprints

The fatigue index during the two blocks of repeated sprints that were embedded during the first half and the second half of the intermittent exercise test are shown in **Figure 11A** and **Figure 11C** respectively. There was a significant main effect of time in both halves (*p*<0.02) indicative of a gradual increase in the fatigue index as the repeated sprints progressed (**Figure 11A and Figure 11C**). However, there was no significant effect of condition and no time x condition interaction effect, and a simple paired t-test of the mean of the fatigue index from all repeated sprints in each half also showed no significant difference between conditions, with Cohens D effect sizes all negligible (first half: *p*=0.86, d=0.01; second half: *p*=0.30, d=0.10; **Figure 11B and 11D).**

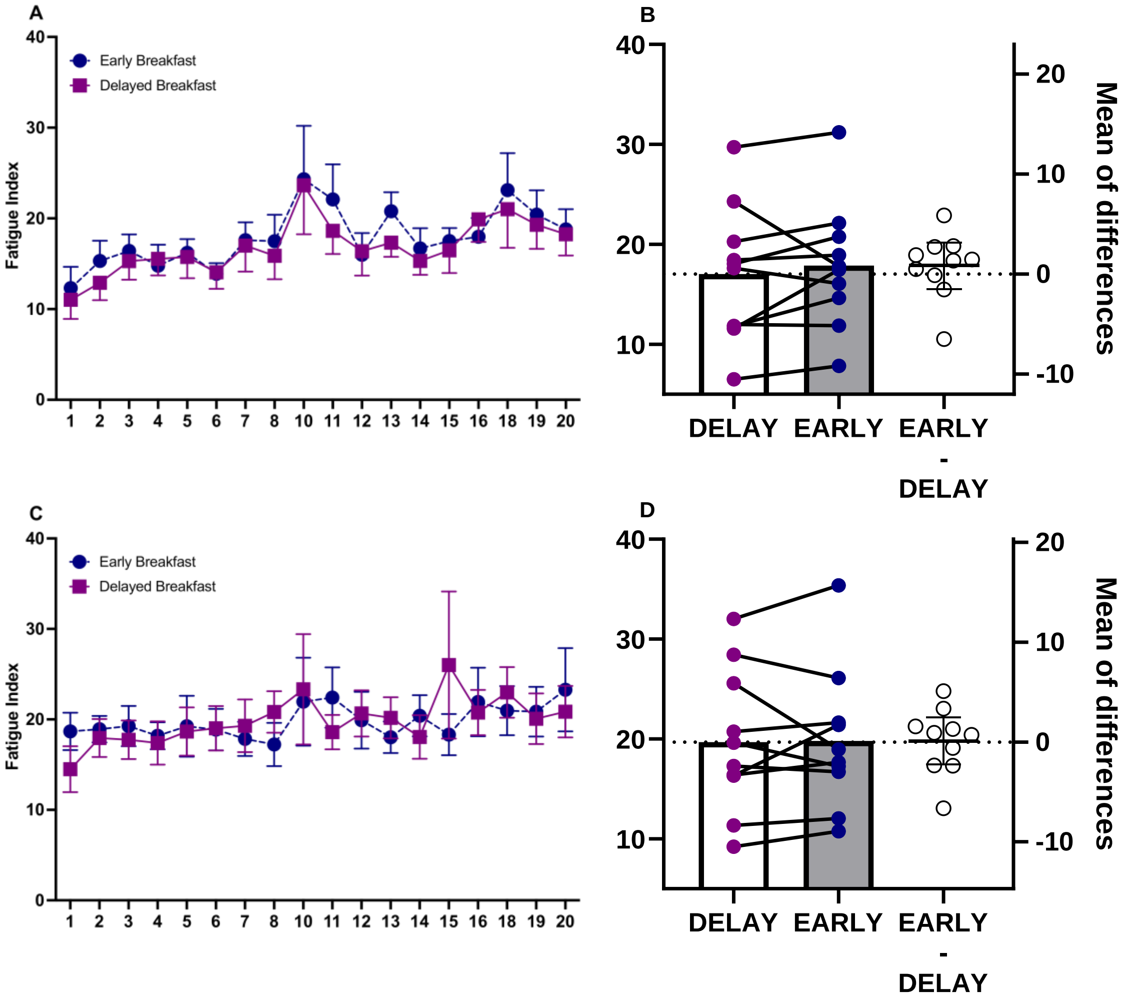

Figure 10: Fatigue Index achieved from each intermittent sprint in the first half (10A) and the second half (10C), alongside the fatigue index averaged across all sprints in the first half (10B) and the second half (10D). Data in 10A and 10C is presented as mean ± SEM. Data on the left-hand axis of 10B and 10D is presented as the mean with individual data points overlaid, whilst the right-hand axis represents the mean difference between conditions ± 95% CI, with clear dots representing individual participant difference scores.

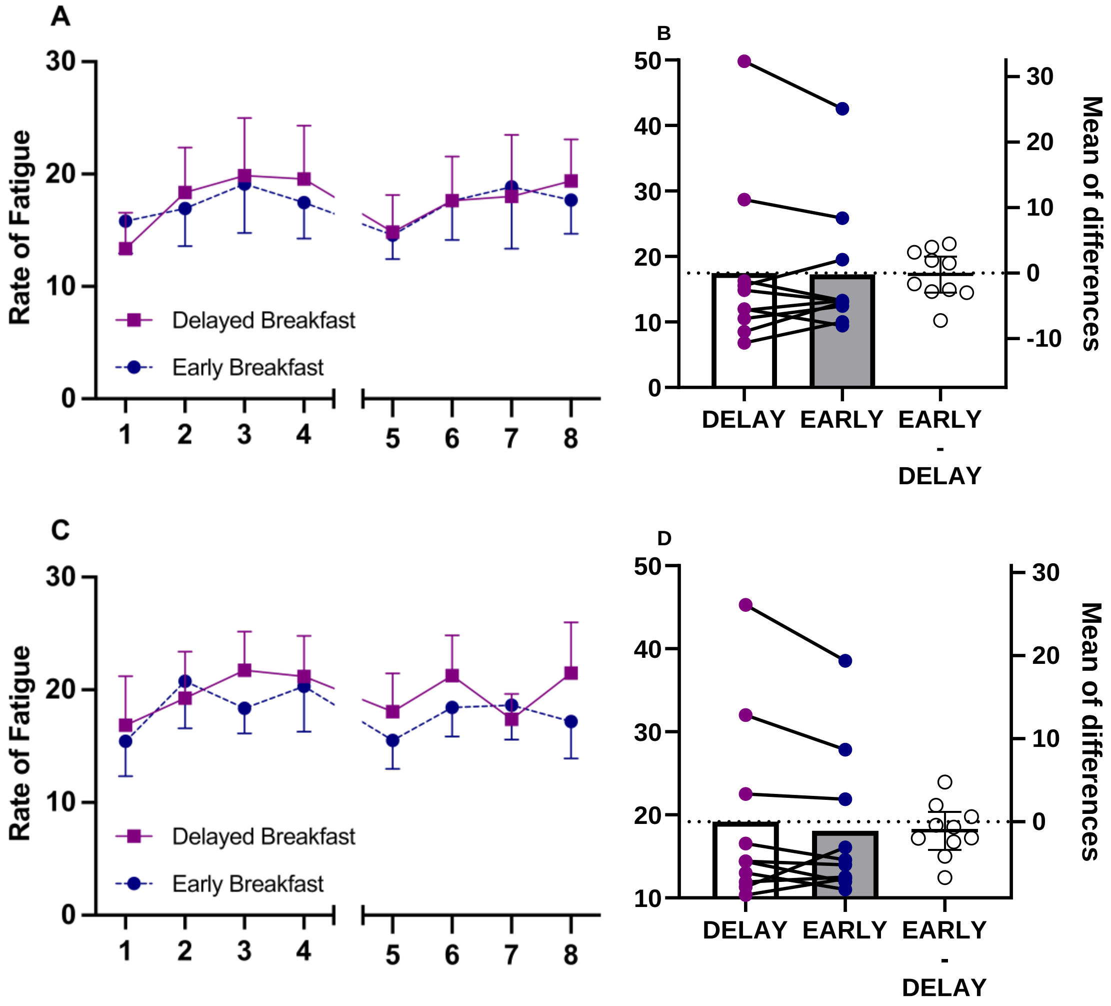

Figure 11: Fatigue index achieved from each repeated sprint in the first half (11A) and the second half (11C), alongside the fatigue index averaged across all repeated sprints in the first half (12B) and the second half (11D). Data in 11A and 11C is presented as mean ± SEM. Data on the left-hand axis of 11B and 11D is presented as the mean with individual data points overlaid, whilst the right-hand axis represents the difference between conditions ± 95% CI, with clear dots representing individual participant difference scores.

Heart Rate During The IST

Participants heart rate during the IST is shown during the first (Figure 12A) and second half (Figure 12B). In both halves, results showed a main effect of time (Both halves *p*<0.0001), however, there was no significant differences between conditions (No main effect of condition) and no time x condition interaction effect.

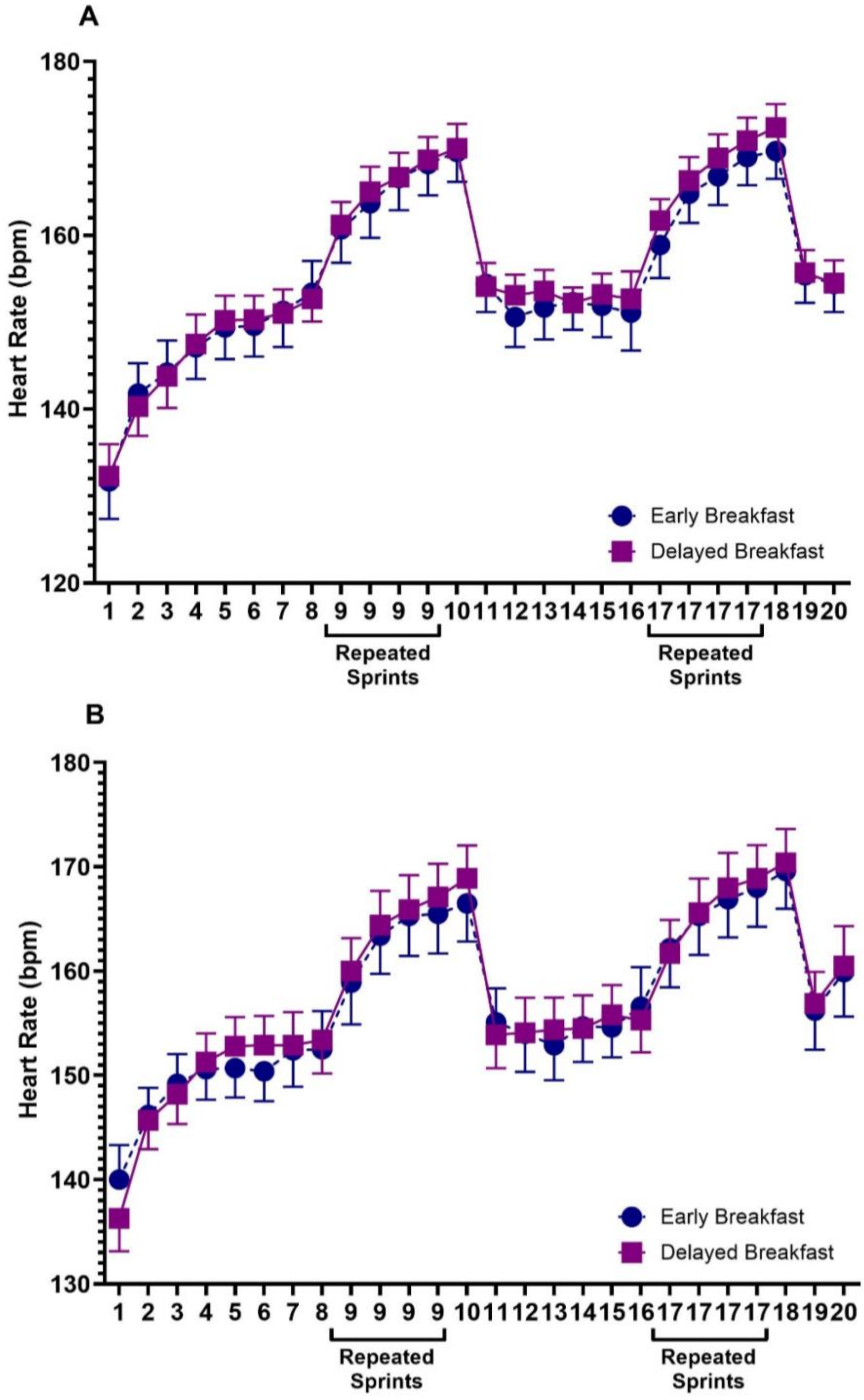

Figure 12: Heart Rate measured after each sprint in the first half (12A) and second half (12B). Data is presented as mean ± SEM.

Rating of Perceived Exertion (RPE) During The IST

Participants subjective ratings of perceived exertion after each sprint is shown below, for the first half (Figure 13A) and the second half (Figure 13B) respectively. In both halves, RPE showed a main effect of time (Both halves *p*<0.0001), however there was not a main effect of condition or a time x condition interaction effect in either half.

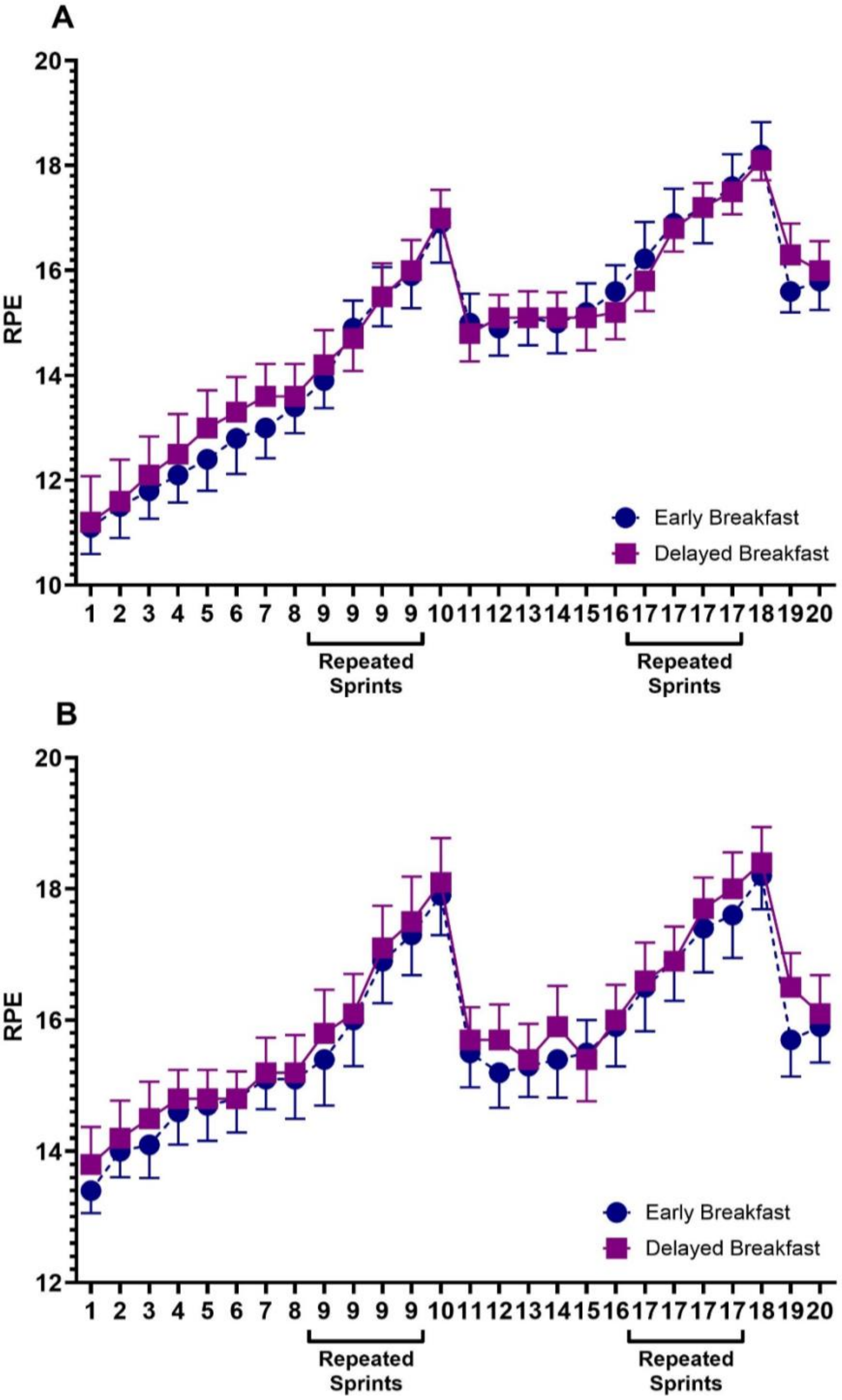

Figure 13: Ratings of Perceived Exertion (RPE) measured after each sprint in the first half (13A) and second half (13B). Data is presented as mean ± SEM.

BRUMS Mood Scale (Pre-Exercise Test)

The results of the BRUMS mood scale completed immediately prior to the exercise are shown in **Figure 14**. There were no significant differences between EARLY and DELAY for any subscales of the BRUMS mood scale, or with the calculated total mood disturbance (Anger: p=0.34; Confusion: p=0.11; Depression: p=0.19; Fatigue: p=0.08; Tension: p=0.26; Vigour: p=0.78; Total mood disturbance: p=0.11).

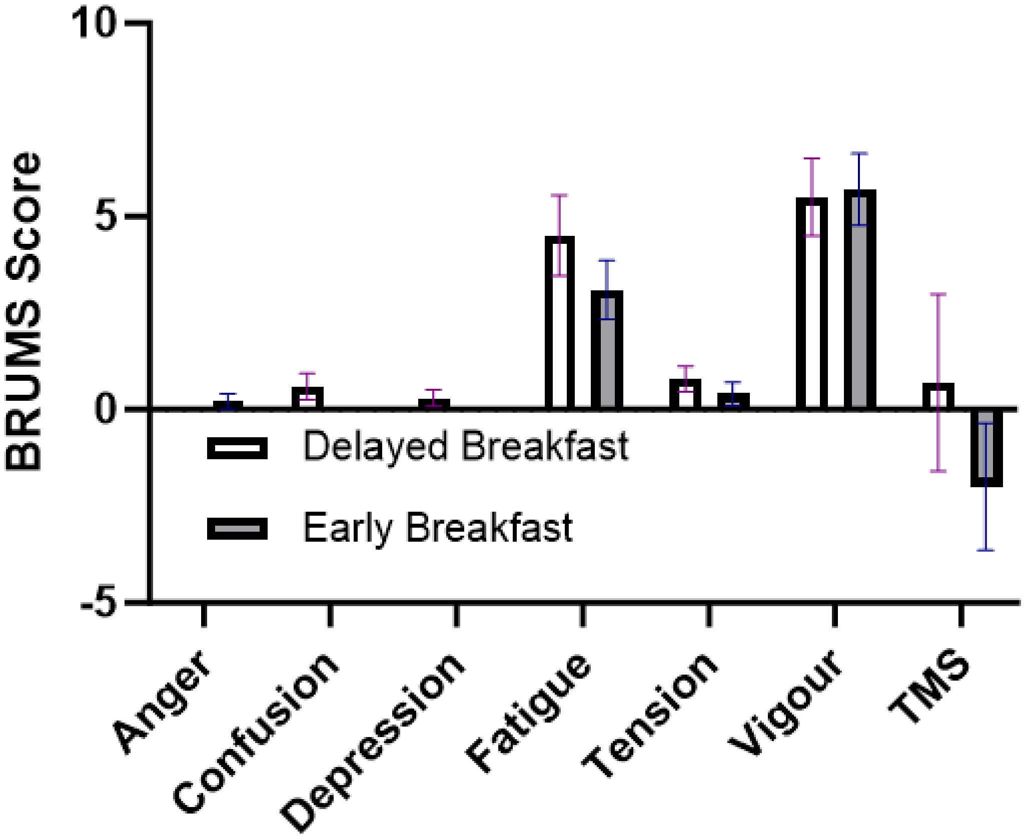

Figure 14: The BRUMS mood ratings, and calculated total mood disturbance, collected prior to the intermittent exercise test. Data is presented as mean ± SEM. Where there is no data shown the mean and SEM were both zero. TMS = Total mood disturbance.

Exit Questionnaire (Manipulation Check)

From the exit questionnaire completed after the conclusion of the study, only one participant could identify a noticeable difference between the two breakfast shakes that were consumed, but they were unable to correctly describe what was specifically different between them. When participants were subsequently informed that only one of the two breakfast meals had contained energy / carbohydrate, and subsequently asked to identify the order in which they thought they had consumed the shakes, less than half of participants guessed correctly (40%), i.e., as would be expected by chance. Together these subjective responses provide evidence of the success of the single-blind placebo control in this study.

Discussion

The aim of this study was to investigate the effect of delaying the timing of a carbohydrate-rich breakfast on afternoon intermittent exercise performance in trained games players using a randomised single blind and placebo-controlled crossover design. We observed no significant difference between the early and delayed breakfast trials in any of the outcome measures of performance from the intermittent exercise test, including peak sprint power, average sprint power, minimum sprint power, or the fatigue index. Crucially, all numerical differences and Cohens D effect sizes for these outcomes between the two trials were also negligible. Taken together, and in contrast to our original hypothesis, the data from this study provide no evidence that delaying the consumption of a carbohydrate-rich breakfast impairs afternoon intermittent exercise performance. This is a novel finding with real-world applications for practitioners and athletes in intermittent sports trying to optimise performance during afternoon training or during competitive match play, as it provides evidence that practitioners can be more flexible in their approach to breakfast (or morning feeding) when preparing for an evening game.

The findings from this study are in contrast to previous studies on breakfast and afternoon/evening performance, which have all provided evidence of a negative effect on exercise performance when omitting, compared to consuming, an early morning carbohydrate-rich breakfast meal. An impairment of performance of ~3-4% has been shown to occur during relatively long duration (~30-min) endurance cycling exercise tasks (Clayton et al., 2015) and by ~1% during a shorter duration (<10 mins) very high intensity rowing time trial (Cornford & Metcalfe, 2018). Interestingly, the impairment of evening performance also appeared to persist when the carbohydrate and energy that is missed due to breakfast omission is subsequently replaced during lunch (Metcalfe et al., 2021). Whilst these three studies have provided interesting and important preliminary evidence on the importance of an early morning carbohydrate rich breakfast meal, there are a several important weaknesses across these studies which may undermine their conclusions. A major aim of the present study was to address these limitations. We firstly utilised a placebo control, to blind the participants to the nutrition intervention, removing the psychological expectations of reduced performance that had been suggested as potential explanations for the results seen in the previous studies above. We also used a highly controlled laboratory design, to counter any potential deviation from the nutritional intervention and to ensure we could be confident that the clamp on energy and macronutrient intake was followed throughout the whole day in both trials, and physical activity levels prior to the exercise task were matched (i.e., no physical activity until the exercise task) across both trials. Finally, we matched our exercise test to the demands of intermittent sports, which can be considered more ecologically valid for the afternoon/evening versus previously researched exercise modes, given it is intermittent sports which are most played in this period. The fact that our findings differ from previous studies suggests that either the effect of breakfast omission on endurance exercise performance does not persist in intermittent exercise performance tasks, or that an improvement in the study design applied has altered the observed effect.

One of the most important limitations of previous studies is the lack of a blinded and placebo-controlled design. Indeed, in all previous studies in the area around breakfast omission and afternoon/evening exercise performance, participants have been aware that they have been either consuming or omitting a carbohydrate rich breakfast. This approach is understandable given the challenges of taste and texture matching a carbohydrate-rich breakfast whilst simultaneously providing the perception of consuming a solid breakfast meal (as would be the case in real-world circumstances). Nevertheless, this raises the prospect of a psychological expectation of improved performance when consuming breakfast vs omitting breakfast, and this may largely explain previous findings, rather than any physiological effect of omitting the breakfast *per se*. Indeed, past studies have referenced this as potential reasoning for their findings (Cornford & Metcalfe, 2018; Metcalfe et al., 2021). The placebo effect, a favourable outcome from belief that one has received a beneficial treatment, has been shown to be an important phenomenon within athletic performance (V. R. Clark et al., 2000). Furthermore, a recent study investigating the placebo effect within sport and nutrition found that when participants ingested either water (WAT), a semi-solid carbohydrate breakfast (CHO) or a taste and texture matched placebo (PLA) in a randomised order, 90 minutes prior to a 10 minute steady state cycle and a ~20 minute cycling time trial to a workload target of 376 kJ, the time trial was completed quicker in both the carbohydrate and placebo trials vs water, however there was no difference in performance when comparing the CHO and PLA trials. Whilst the placebo and carbohydrate shake were administered in a double-blind manner, with subjects told the study was researching the effect of two breakfast drinks, it was impossible to blind either the experimenters or participants from the water trial. Blood glucose levels showed a significant time, trial and time x trial effect, with a clear rise following feeding in the CHO trial that was not seen in the PLA and WAT trials, with RER and CHO oxidation also greater in the CHO trial vs PLA and WAT. Given this, and the performance results shown, it raises the potential of there being a placebo effect of ingesting breakfast prior to exercise, suggesting that for this exercise mode and intensity, nutritional intake may be of psychological rather than physiological benefit (Mears et al., 2018b). A further study using a similar study design to the this study – participants consumed either water, a semi-solid carbohydrate meal or a taste and texture matched placebo meal – but looking at the effect on resistance exercise, in the form of four sets to failure at 90% of their 10 repetition max of both the back squat and bench press, found that more total back squat repetitions were completed in the carbohydrate and placebo than water, but with no difference observed between the carbohydrate and placebo trials, and no difference in any of the trials for total bench press repetitions (Naharudin et al., 2020b). This appears in agreement with the findings of the current study.

The placebo effect continues to showcase itself when looking more closely into breakfast consumption alone, with a randomised, participant blinded study showcasing that a very low energy, viscous placebo breakfast, with a volume equating to 5% mL/kg body mass, suppressed appetite scores similarly to a standardised breakfast meal equating to 20% of total daily estimated energy requirements, with both suppressing appetites significantly more than a water control (Slater et al., 2022). Whilst *ad libitum* lunch intake was only suppressed in the breakfast consumption trial, the placebo trial still had a lower cumulative energy intake than in the food trial, providing valuable insight that a placebo breakfast can attenuate subjective appetite sensations associated with breakfast omission, despite containing very low levels of energy. Our study agrees with the subjective appetite findings shown here, adding to the body of evidence that suggests that in habitual breakfast eaters, a pre-exercise (consumption 90 minutes prior) breakfast meal likely influences exercise performance via a psychological effect rather than a physiological effect, when participants are completely blinded to what they are consuming.

We utilised a similar semi-solid viscous placebo breakfast to a previous study investigating how the perception of breakfast ingestion can enhance exercise performance (Mears et al., 2018). Importantly, the blood glucose, substrate oxidation and appetite responses over the course of the two trials, alongside the responses to the exit questionnaire, suggest that our single blind placebo-controlled design was successfully implemented. Specifically, following the first shake at 8am, blood glucose concentrations rapidly increased in the early breakfast trial but remained stable in the delayed breakfast trial, whilst there was also a shift to higher CHO oxidation in the early compared to the delayed breakfast trial. Crucially, alongside this, there was a clear suppression of appetite seen in both trials after each meal, followed by a gradual increase in appetite up to the following meal, despite one of these meals in each trial containing no energy or carbohydrate. In fact, there were no significant differences in appetite ratings between the two trials throughout whole of the morning and afternoon of the trial. Together these responses can be interpreted to mean that participants perceived they were consuming a breakfast meal at both morning time points, despite the different pattern of energy and carbohydrate intake at these meals. It should be noted, however, that our exit questionnaire was completed following the conclusion of the entire study, and this may be a limitation as participants could well have not been able to well recall the taste of texture of the shakes during trial 1, limiting the accuracy of answers to the questionnaire. When considering exit questionnaires, the use of a sensory style questionnaire may have provided a more robust option, however a drawback to this would have been the potential for participants to become suspicious of the trial and begin to realise something was different across conditions. The study authors wished to ensure that the placebo remained completely unexposed, hence the use of a written questionnaire only once the full study was concluded.

However, when looking at the blood glucose data alongside the CHO and fat oxidation data, a potentially interesting finding emerges. When comparing the substrate response for the first nutritious meal of the trial (8am for EARLY, 10am for DELAY) the blood glucose response was not as pronounced in DELAY compared to EARLY, with CHO oxidation also trending lower and fat oxidation trending higher. Specifically, the two hour total area under the curve for blood glucose, CHO oxidation and fat oxidation for following the high-CHO breakfast, was lower in the delayed breakfast condition. This suggests that the placebo shake provided in DELAY reduced the glycaemic response to the high carbohydrate shake provided at 10am, a finding which could have potentially beneficial implications to both the recreational exerciser, as well as those living with chronic conditions such as type 2 diabetes. The mechanisms for this finding are unclear, but a potential explanation could be related to the second meal effect, whereby the consumption of a prior meal has been shown to improve glucose tolerance to a subsequent second meal (Clark et al., 2006). Whilst limited, there is small evidence to suggest that a placebo meal can reduce plasma glucose area under the curve by 28% versus a water control (Sievenpiper et al., 2007), and it could be suggested in the current study that having a preload prior to the high carbohydrate shake, however minimal its nutritional contents were, allowed the second meal to exhibit a reduced glycaemic response. However, a more likely explanation may be related to a delay in gut glucose absorption, due to the presence of the contents of the carbohydrate shake, particularly Xantham gum, which has been shown to be able to suppress blood sugar levels and slow glucose absorption in the digestive tract after consumption (Fuwa et al., 2016; Osilesi et al., 1985). This could be an important finding, as it may show that individuals are able to utilise the second meal phenomenon but with a reduced carbohydrate preload. For those with diabetes, this could be useful with regards to improving the artificial inducing of the second meal effect as a therapeutic treatment (Chen et al., 2010), by potentially helping to reduce the glycaemic load of the initial meal/delaying glucose absorption in the digestive tract to help manage blood glucose levels throughout the day. These findings may also have relevance for the recreational exerciser who is interested in improving cardiovascular and metabolic health.

Given the importance of the placebo effect in other contexts (Burke et al., 2000; V. R. Clark et al., 2000; Hurst et al., 2020; Naharudin et al., 2020b; Ross et al., 2015), and the accumulating evidence that the effect of breakfast on morning performance is driven by a placebo effect, our interpretation is that the placebo-controlled and single blind design utilised in the present study is this is the most likely explanation for different findings between our study and previous studies on breakfast and afternoon exercise performance. It is therefore important that future research studies replicate the previous research in this area (Clayton et al., 2015; Cornford & Metcalfe, 2018; Metcalfe et al., 2021) to understand whether a placebo control, single or double blinded study design would change the results observed on other exercise modes to no effect, or if there is still a prevailing physiological concept behind breakfast omission/manipulation and afternoon/evening exercise performance. This rationale is further supported by the responses to our exit questionnaire, where 9/10 participants reported they were unaware there was a difference between the meals between trials, and they could not accurately report the order in which they received the two trials when the differences were pointed out to them.

An alternative possible explanation for the lack of effect in this study compared with previous studies, is the difference in exercise mode. No study investigating the effects of breakfast upon afternoon and/or evening exercise performance has used intermittent exercise. This is important and somewhat surprising given its greater applicability to the real-world, due to its commonality in being played in the afternoon/evening compared with previously researched exercise tasks. Intermittent exercise demands, particularly in team sports such as football or rugby, whereby work rate can depend upon self-chosen exercise intensity and the prevailing demand against the whole team, have seen comparatively less research compared to more individual sports such as cycling and marathon running (Reilly et al., 2008). Whilst players often travel 10-13km in intermittent team sports, the majority of this is walking and/or low-intensity running, which require limited energy turnover (Bangsbo et al., 2006). Compared to a 2000m row, as was used in Cornford and Metcalfe (2018), the metabolic demands are much different. In a typical 6-minute rowing race, there is an initial 1-minute period largely dependent on anaerobic metabolism, before a longer 4-minute period of very high steady state aerobic metabolism, before a further highly anaerobic period in an all-out sprint to the finish, which is a unique, metabolically diverse race pattern not often seen in other sports in such a short period (Kyparos et al., 2009). Therefore, aside from the first minute, rowers have been found to be near to or at their maximum oxygen uptake for the entirety of the 5–7-minute trial (Das et al., 2019). It has been found that throughout a 2000m rowing time trial, the aerobic energy system contributes 77%, through muscle glycogen degradation, towards satisfying the energy demands, with the anaerobic energy systems contributing 33%, through phosphates, ATP and phosphocreatine (Martin & Tomescu, 2017). In comparison, whilst the aerobic energy system is highly taxed during intermittent exercise, with average oxygen uptake around 70% (Bangsbo et al., 2006), the oxygen demand is reduced in intermittent exercise, showing a clear difference in the energy systems being utilised. In intermittent exercise, the dependence on muscle glycogen may be somewhat reduced, due to the periods of walking and/or low intensity running, where there is a more substantial contribution from fats to the energy demand (Essén et al., 1977). Whilst both exercise modes show a high level of dependency on muscle glycogen, and its degradation causes fatigue to set in (Costill & Hargreaves, 1992), it could be suggested that intermittent exercise oxidising fat to meet a portion of the energy demand during the low to moderate intensity portions of the exercise mean that the nutritional timing manipulation will have had less effect in the present study versus previous studies, given the focus on providing a high-CHO breakfast.

Previous studies have completely omitted a morning carbohydrate-rich breakfast meal and compared it to an early morning meal. It could be argued that this sort of dietary manipulation is one that is unlikely to be recommended by practitioners in a real-world performance context. However, any recommendation by practitioners appears to encounter challenges with adherence (D. E. Anderson, 2010; Cole et al., 2005) -

Athletes sometimes simply do not follow what they are told, despite being informed of its benefits. Dietary practices for athletes on competition/matchdays have been shown to be poor, with consistent reports of lower macronutrient and caloric intake than is recommended for athletes (Gamage & De Silva, 2014; Nepocatych et al., 2017; Sandra Maria Lima Ribeiro et al., 2015; Zanders et al., 2021). The reasonings behind this lack of adherence to recommended macronutrient, particularly carbohydrate, intake has revolved around athletes reporting game-day stress and anxiety, including an expectation to perform well and nervousness around encountering GI issues during competition, making it a real challenge practically to encourage athletes to consume a pre-match/gameday meal meeting the CHO targets recommended by scientific evidence (Holway & Spriet, 2013). This must be considered when thinking about the practical implications of the present study, with continued education and interventions from qualified practitioners vital to aid in driving home the importance of optimising nutrition on gameday performance. This may even extend to education of coaches in the high-performance environment, with research suggesting that athletes can find the environment a coach creates a stressor, with a “slim to win” mentality creating barriers when it comes to meeting macronutrient targets, and this could help improve overall adherence to nutritional interventions set by practitioners (Bentley et al., 2021).

In the present study we therefore opted to delay rather than completely omit breakfast. This difference in approach could well explain, at least in part, the lack of effect in this study compared to previous studies. In the most current study in this area prior to the present study, whilst there was a clamp on total nutritional intake, this was done by providing participants with a large lunchtime meal to compensate for the lack of caloric intake at breakfast. In real terms, this provides two windows for glycogen synthesis, with a four-hour window in the EARLY trial, and a smaller, two hour window in the DELAY trial. In comparison to previous trials, there has been no two hour window as breakfast has been completely omitted until lunch, extending the overnight fast, or provides a four hour window if consuming breakfast. Blood glucose and serum insulin levels after a high-CHO breakfast meal appear to peak at 60-90 minutes after consumption (Jovanovic et al., 2009), which broadly agrees with our present study where blood glucose levels spiked above baseline measurements for 30-90 minutes. Furthermore, the study demonstrated increased glycogen synthesis in the skeletal muscle after a standardised lunchtime meal when consuming a high-CHO breakfast versus omitting breakfast, with a 50% greater incorporation of dietary glucose into muscle glycogen two hours post lunch (Jovanovic et al., 2009). The data from this study suggests that insulin secretion after the breakfast meal suppresses plasma free fatty acids (FFA), facilitating improved post-lunch carbohydrate economy and therefore allowing greater muscle glycogen storage. It is known that the level of muscle glycogen prior to exercise is a determinant for optimal performance (Saltin, 1973) and therefore this may have had implications in the previous breakfast omission studies, whereas our present study, whereby both trials consumed a high-CHO breakfast meal at least two hours before lunch, may have negated any performance benefit this could have elicited, hence seeing no difference across EARLY and DELAY.

Meals of larger weight/volume and caloric content have been shown to increase gastric emptying times and the rate of acceptance of energy by the duodenum (Hunt et al., 1985; Moore et al., 1981). In the present study, the breakfast meal was a semi-solid shake containing maltodextrin, which has been shown absorb and digest at a high rate, similar to that after ingestion of pure glucose, further backed up by comparable post ingestive insulin response rates to glucose (Hofman et al., 2016; Jeukendrup, 2010). The composition of the breakfast meal being semi-solid, rather than solid, will also have caused greater gastric emptying in comparison to previous studies which all utilised solid food in their interventions. In a previous breakfast omission study by Metcalfe et al., (2021), one participant reported feeling of lethargy post lunch in the breakfast omission trial, whereby they had consumed a large lunchtime meal consisting of 50% of their estimated daily energy intake, as per the study protocol of clamping energy intake. Here, we have removed this as a possibility whilst maintaining a caloric clamp, by delaying the breakfast meal timing, rather than omitting and adding extra calories at lunchtime to compensate. Whilst speculative, this spreading of calories through the day rather than loading the energy intake into what is effectively a pre-match meal, may well have caused us to have different findings to other studies in this area. The use of a semi-solid breakfast meal, and delaying consumption rather than omitting completely, ensures that muscle and liver glucose were replenished more quickly after the overnight fast, therefore limiting the likelihood of the conclusions from previous studies having application for the present study.

It is also important to point out that we were only able to complete 10 participants in this study which is lower than the 16 participants we calculated were needed to provide 80% power to detect a similar effect size for the performance difference between breakfast omission and consumption trials in two previous studies (Cornford and Metcalfe 2018; Metcalfe et al 2021). Therefore, it is possible that our finding of no effect of delaying a carbohydrate rich breakfast on afternoon intermittent performance is the result of low statistical power and a type 2 statistical error. However, the negligible numerical mean differences and effect sizes for all performance outcomes suggests that this possibility is unlikely and that we would observe similar findings with a larger sample size. The reliability of the intermittent sprint test used for the study is also an important note, as it is a test adapted solely from a previous paper by Bishop & Claudius (2005) investigating the effect of induced metabolic alkalosis on prolonged intermittent sprint performance. However, a prolonged cycling intermittent sprint protocol using a cycle ergometer has been shown to be a reliable measure of exercise performance, particularly when using peak power output as a metric/performance outcome (Hayes et al., 2013).

The data from this study has practical implications for intermittent sport athletes. The conditional demands of intermittent sports, such as football, have increased dramatically in recent years, particularly the high-intensity element of the exercise (Gomez-Piqueras et al., 2019) and as such, practitioners are constantly looking to innovate and find new ways to help delay the onset of fatigue (Williams & Rollo, 2015). The results from this study show for the first time that from a physiological perspective, the nutritional timing of the breakfast meal does not impact on intermittent exercise performance in the late afternoon/evening period. However, given the results from previous studies which differed with the research findings from the present study, albeit using a different approach (fed vs fasted rather than early vs delay) further research is required with other exercise types whilst utilising a placebo and control trial, to determine to what extent to which psychology plays a part in any impacts to performance. This would seem to be a logical next step in the research area given the study conducted by Mears et al. (2018) which introduced a water control alongside the semi-solid placebo and still found that a 20-minute cycling time trial was completed with no significant performance advantage vs a semi-solid carbohydrate meal, but both were significantly greater than a water control. The suggestion from this was that the improvement in performance was attributable to a psychological effect, which is a similar conclusion to what this study considers the most likely reason for our results. Therefore, delving deeper into this area and trying to quantify to what level psychological and physiological mechanisms interact to produce these results should be a focus of future studies. With another key difference of this study versus past research, both in breakfast and evening performance as well as placebo studies, being the mode of exercise, physiology could also be an important aspect. The exercise test used involved repeated 6-s exercise periods interspersed with periods of lesser intensity. Research has shown that repeated exercise periods as small as 6-s can still have a relatively high aerobic contribution (Balsom et al., 1999). Compared with the Mears et al. (2018) test, which used a steady state exercise followed by a shorter, self-paced time trial, this study utilised a longer duration exercise test, whereby glycogen availability is known as a limiting factor (Sherman et al., 1991) and despite the lack of a water control, this study showing similar findings to Mears et al. (2018) reflects a novel finding, showing that even when glycogen availability is a known limiting factor, a placebo control still negates this.

For high level athletes, getting the correct balance on a matchday/competition day requires a multitude of factors, with nutrition a large part of this. However, other factors must be considered by the athlete and/or support staff, including but not limited to sleep, hydration, training load, recovery cycle, previous match/competition outcomes and perception of upcoming opponents (Sansone et al., 2021). The results from this study, however, show that practitioners and/or athletes can be flexible with their breakfast meal, when performing later in the day, and can fit in with the needs of the athlete and team, so long as the generic recommendation that athletes continually consume a CHO rich diet. This advice seemingly needs no further, more specific guidance on timing beyond the currently well researched 4-hour pre-exercise window (Kerksick et al., 2017), although the aforementioned challenges around athletes' nutritional practices on game days/competition must be recognised. The main limitation of this study was its lack of a true control trial, so adding in a water only trial, which would have allowed us to provide a definitive answer on the placebo effect that we speculate is the reasoning behind the results found, should be a goal of future studies. Future studies should look to address this by using a similar approach to the placebo study from Mears et al., (2018), with the addition of a water only control trial to this study design to aid in confirming our findings. A further limitation to this study was a lack of reporting in the days prior to the test taking place, i.e. 48-72 hours prior. Whilst the study asked participants to record the 24 hours prior to the testing days, we did not have an understanding or record of participants diet in the 2-3 days prior. Studies have previously shown that consuming a high-CHO diet 48 hours before intermittent running has enhanced performance vs a control diet which did not meet nutritional guidelines for carbohydrate (Bangsbo et al., 1992). A further study showed a high carbohydrate diet consumed for 3 days prior to a maximal interval test (MIT) improved total work done by 5.8% versus a low carbohydrate diet not meeting nutritional guidelines (Jenkins et al., 1993). It could therefore be seen as a limitation that the study did not track diets beyond 24 hours, because this could have had on exercise performance. Although, it should also be considered that the study itself was already heavy with regards to participant burden, and this had to be minded when considering the study design. However, there is no doubt that extending the monitoring of the diet would have improved the study, and this should be considered in future studies looking to advance the research area. It should also be considered that whilst repeated sprint cycling performance has shown strong correlations with repeated running performance, the specific test used has not been validated against external tests of intermittent performance, such as the Loughborough Intermittent Shuttle Test (LIST), and future studies may wish to investigate this further to ensure that the exercise test used best reflects prolonged, real-world intermittent exercise performance.

Studies might also wish to focus on whether similar findings can be obtained in other exercise modes when a placebo and control trial is used to eliminate any psychological reasoning for the findings. Where possible, this placebo control should utilise a solid meal, to improve ecological validity, and could look to investigate high vs low carbohydrate meals, or high GI vs low GI to further investigate how granular recommendations around breakfast for afternoon/evening exercise performance need go.

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