The effect of mouth rinse and ingestion of carbohydrate solution on short intensive exercise – How can we explain the increase in exercise performance?

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1.0 Abstract

Background: Ingestion of carbohydrates during exercise in a fasted state has been shown to improve high-intensity exercise performance. The mechanism responsible for the improvement remains uncertain. Recent studies suggest that rinsing the mouth with a carbohydrate solution improves performance in the latter stages of high-intensity exercise without changes in circulating glucose levels. There has also been an absence of a peripheral metabolic action of exogenous carbohydrates and thus central effects have been postulated to explain this phenomenon. **Aim:** The purpose of the present study was to investigate whether there were individual and/or additive effects of carbohydrate mouth rinse, fluid intake and carbohydrate ingestion on 1-h time trial cycling performance. The project further investigated the response in circulating markers of fuel utilization. **Methods:** Eight recreationally trained cyclists volunteered for this randomised, counterbalanced, double-blind study. After a preliminary familiarisation session, four main trials were performed on an electronically-braked cycle-ergometer with each trial separated by 7 days. Each main trial took place over two days. On Day 1 the participants underwent a 90 min glycogen reducing exercise protocol, immediately followed by a low carbohydrate meal and then a subsequent overnight fast. The following morning a 1-h time trial performance test was conducted. Subjects performed a certain amount of work as fast as possible for the performance test. The main trials included a 15% carbohydrate mouth rinse (CHOR), ingestion of a 7.5% carbohydrate solution (CHOI), a placebo mouth rinse (PLAR) and placebo ingestion (PLAI); solutions were administered every 12.5% of exercise completed. Blood samples and perceptual measures (perceived activation, pleasure-displeasure and ratings of perceived exertion) were taken every 25% of exercise. A profile of mood states questionnaire was also administered prior to the time trial and immediately post exercise. **Results:** There were no significant differences in performance time between treatments (P=0.55). However, there was a main effect of treatment for power output (P=0.002) with higher values in CHOI (231.4 ±9.8 W) relative to other trials (222.1-224.6 W; P<0.05). Plasma glucose was higher in CHOI at 75% (5.4 $mmol \cdot L^{-1}$) and 100% (5.9 $mmol \cdot L^{-1}$) of the time trial relative to other trials (3.9-4.7 $mmol \cdot L^{-1}$; P<0.05). There was a main effect of treatment for insulin (P=0.001) with highest values in CHOI (5.14 $mmol \cdot L^{-1}$) relative to the other trials (4.2-4.7 $mmol \cdot L^{-1}$; P<0.05). There were no significant differences reported between treatments for any of the perceptual measures. **Conclusion:** Ingestion of a carbohydrate-electrolyte solution was associated with a decrease in performance time during a 60-min cycling performance time trial in comparison with CHOR, PLAR and PLAI in a glycogen reduced state. This suggests that peripheral and not central effects are largely influenced by the use of a carbohydrate supplement.

Keywords: *fatigue, endurance performance, ergogenic, supplementation, central, peripheral, metabolism, fluid intake*

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2.0 Introduction

It is common practice for athletes from different sports to ingest carbohydrate-electrolyte solutions for performance purposes. The ergogenic effects of carbohydrate intake during prolonged exercise have been well explained (e.g. Jeukendrup et al, 2008). Research in this area was originally conducted to examine the role of high muscle glycogen stores pre-exercise and its effect on exercise performance (Bergström et al, 1967; Hultman et al, 1967), however, in the last 20 years research has been focussed on ingestion of carbohydrates before and during exercise (Jeukendrup et al, 2010).

During hard muscular work as seen during endurance exercise there are a number of performance-limiting factors categorised under the complex area of fatigue. There are many potential reasons for fatigue during endurance exercise and lack of metabolic fuel is one of these aspects for example glycogen depletion (Costill et al, 1977). Fatigue has been related to the inability of muscle to maintain a specific level of contraction and the suggested causes may lie in either the central nervous system, the final motor neuron, the neuromuscular junction or the muscle (Brooks et al, 1985).

Central fatigue has been defined as a 'negative influence that exists despite an individual's full motivation' and has been associated with feelings of tiredness, lethargy and also in the perception of pain (Davis et al, 1997). Fatigue is a multidimensional phenomenon and relates to the environment, the type of exercise itself and the individual's training and physiology (Brooks, 1985). A number of theories exist as to what constitutes 'central fatigue' and as a result, further research is needed. Fatigue may involve a depletion of energy supplies or an accumulation of waste products, changes in pH, depletion of nervous system transmitters and dehydration thus relating to the inability of muscle to maintain a specific level of contraction (Kraemer, 1983).

Research into fatigue has prompted further speculations into fuel utilisation for example carbohydrate ingestion and its effect on performance in relation to delaying the onset of fatigue. Much of the previous research surrounding endurance exercise has found that carbohydrate ingestion during exercise can increase endurance capacity (exercise to volitional fatigue; (Bishop et al, 2002; Coyle et al, 1992). There are fewer studies examining the influence of ingestion of carbohydrate-electrolyte solution on endurance performance; a more

accurate measure of performance as it does not merely measure time to fatigue but also assesses a number of measures that have been associated with performance (Jeukendrup et al 2008). Thus, recently, endurance performance based tests have currently been the centre of interest (Jeukendrup et al, 2008; Jeukendrup et al, 2004; Jeukendrup, 2010).

Speculations regarding the improvements in performance following carbohydrate-electrolyte ingestion include better maintenance of blood glucose, increase in carbohydrate oxidation and/or more efficient sparing of muscle glycogen during intermittent and continuous high-intensity exercise (McConell, 1999; Coggan, 1988; Winnick et al, 2005). Tsintzas et al (1995) examined carbohydrate ingestion and its effects in glycogen utilisation in different muscle types. The authors suggested that the improvements in exercise performance observed in research with carbohydrate ingestion may be due to an increase in sparing of muscle glycogen. However, Beelen et al (2009) reported no improvements in cycling performance when participants were in a fed state with carbohydrate ingestion. The authors speculated that when liver glycogen stores are available and endogenous glucose levels are not compromised it seems there is an absence of ergogenic effects upon carbohydrate supplementation and performance. This suggests that accessing the proposed small metabolic changes observed with carbohydrate supplementation in a post-prandial state may not be appropriate. However, in this study they failed to examine blood glucose in their participants which may have clarified their findings to some extent and more research is needed with fasting type protocols in order to help amplify these small changes.

There have also been reported improvements in performance by delaying impaired central nervous system (CNS) function (Nybo et al, 2003; Winnick et al, 2005). However, the underlying mechanism to explain the increase in performance was not clear. Carter et al (2004a) conducted an experiment with the above theories in mind. Cyclists performed a 40 km time trial where, in one trial, a glucose solution was infused and in the other trial a saline solution was infused. Blood glucose concentrations and glucose disappearance were twice as high in the glucose infused trial however, there was no effect on performance even though it was thought that glucose had been taken up and oxidised in the muscle (Carter et al 2004a). Moreover, as there was no clear metabolic explanation for enhanced performance from carbohydrate ingestion centrally-mediating mechanisms were also thought to be present and Carter et al 2004a suggested that further exploration is needed in this area.

Recently, carbohydrate feeding has been found to improve performance during shorter durations and of higher intensity exercise (Jeukendrup et al, 2010; Anantaraman et al, 1995). It is thought that during higher intensity exercise, plasma glucose concentrations do not decrease, as there is sufficient glycogen to sustain the short duration of exercise (~60 min) (Jeukendrup, 2010). However, in the research speculating this, there was failure to examine blood glucose concentrations and there was no attempt to analyse the effect of carbohydrate ingestion in the glycogen reduced state to clarify these points. In fact, Romijn et al (1993) speculated that in short duration, high intensity exercise, carbohydrate oxidation rates are high and are likely not influenced by ingestion of carbohydrates depending on the increasing intensity of exercise and thus the theory of a central mechanism was brought to researchers' attention again.

Although there is plentiful research showing the benefits of carbohydrate ingestion on performance there has also been reports of gastrointestinal tract (GI) discomfort which has led to ergolytic effects on performance (Rehrer et al, 1991; van Nieuwenhoven et al, 2005). Research has examined the effects of carbohydrate ingestion and GI discomfort on performance and have shown that performance decreased with the presence of GI discomfort (van Nieuwenhoven et al,2005). The discomfort that is experienced by many people during exercise from carbohydrate ingestion, has called for further research in carbohydrate use and its association with exercise performance. With questions revolving around the exact mechanism of carbohydrate intake on endurance performance, and problems of GI discomfort associated with carbohydrate ingestion, recent research in rinsing the mouth with a carbohydrate solution has developed.

Rinsing the mouth with a carbohydrate solution has shown a similar effect in improving the later stages of high-intensity exercise without any changes on circulating glucose levels and absence of a peripheral metabolic action of exogenous carbohydrates (Carter et al, 2004b; Pottier et al, 2008; Rollo et al, 2008; Chambers et al, 2009). Functional magnetic resonance imaging (fMRI) research has demonstrated that neural activity is activated at two different times with ingestion of glucose (Liu et al, 2000). During a 48 minute continuous functional scan, glucose was ingested at the 10 minute point. The first peak in activity was evident immediately after the glucose was ingested and the second peak occurred 10 minutes later and was related to plasma glucose changes, after intestinal absorption (Liu et al, 2000). With these findings in mind Carter et al (2004b) performed one of the first studies examining the

effects of a carbohydrate mouth rinse on cycling performance. The authors found that mouth rinsing with a carbohydrate solution improved endurance performance by 2.9% when compared to the placebo solution. The mere presence of carbohydrate in the mouth has been postulated to influence endurance performance by 'central factors' as a carbohydrate mouth rinse may involve stimulation of cortical taste neurons (Carter et al, 2004b); these receptors are thought to exist with neuronal communication to pleasure centres in the brain (Jeukendrup et al, 2008). Evidence in this area is lacking at present, however, researchers understand that the brain can sense certain composition changes in the mouth and it has been suggested that activation of these taste-related brain regions can influence emotion and behaviour (Kringelbach et al, 2004). Therefore, this may have an impact on exercise performance.

Prior to examining the effects a carbohydrate mouth rinse has on performance there have been observations on the effects of ingestion of fluid compared to no fluid on endurance capacity. A study reported that during an endurance running capacity test, participants showed an improvement in performance when compared to the no fluid trial (Fallowfield et al, 1996). A later cycling study reported that the improved endurance capacity when fluid was ingested may be a result of a reduced heart rate, core temperature, and also the utilization of muscle glycogen compared with cycling without fluid ingestion (Hargreaves et al, 1996). The comparison between rinsing with a carbohydrate relative to rinsing placebo and ingestion of a placebo has not previously been examined in the same experimental protocol and thus the relationship between the postulated carbohydrate mouth rinsing central effects may not be as beneficial as it has been speculated. Further research is needed to explore these areas in more depth and this could be analysed though a Latin square design type protocol where variation can be controlled in two directions.

There has also been conflicting evidence suggesting that mouth rinsing and ingestion of carbohydrates do not always support performance improvements (e.g Whitham et al, 2007; Beelen et al, 2009; Rollo et al, 2011). In many of these cases there was a lack of perceptual measures including the effect of carbohydrate mouth rinse and ingestion on mood, and little endocrinal and metabolic measures have been examined to assess performance despite the hypothesised 'central effects'.

Previous research has failed to identify the mechanism behind the ergogenic effects associated with carbohydrate ingestion and carbohydrate mouth rinsing. While the exact mechanism remains elusive, the debate between the central versus peripheral effects of carbohydrate supplementation continues. There have also been limited studies that have analysed carbohydrate mouth rinsing versus carbohydrate ingestion (Gant et al, 2010; Rollo et al, 2011). Most of the previous research has analysed the effects on performance with a carbohydrate mouth rinse versus a placebo mouth rinsing and in some of these studies (Beelen et al,2009; Carter et al, 2004b; Pottier et al, 2008; Rollo et al, 2010; Rollo et al, 2008; Whitham et al, 2007), participants have also in some cases not being blinded between treatments suggesting that there may have been participant bias between treatments. There has also been a lack of data collected to explain these theories, for example there has been very limited endocrinal data analysed (Jeukendrup et al 2008; Rollo et al 2008; Rollo et al 2010), limited oxygen uptake measurements (Pottier et al 2008; Rollo et al 2010), and limited perceptual measurements such as mood changes (Gant et al 2010; Jeukendrup et al 2008; Whitham et al 2007). Carbohydrate supplementation has been associated with 'feel good' sensations (Backhouse et al 2007). The question must then be addressed, if mouth rinsing is associated with central effects why has mood not been assessed in detail before and why has previous research not attempted to analyse all of the central and peripheral associated measures at once? Chambers et al (2009) examined the effects of carbohydrate mouth rinsing through fMRI and found that both glucose and maltodextrin activated regions of brain (insula/frontal operculum, orbitofrontal cortex and striatum) associated with reward which are capable in mediating behavioural responses. However, in this study the swill expectorate was not weighed afterwards and therefore it cannot be certain that some of the solution was indeed swallowed.

As noted earlier, none of the previous research has attempted to analyse the central and perceptual effects of mouth rinsing versus ingestion of a carbohydrate solution in the fasted state. New research is in need to develop and explain these speculations further. In order to examine the central and peripheral – such as fuel provision effects of carbohydrate ingestion and carbohydrate mouth rinsing on endurance, a lab based Latin square design is needed. This should include the treatments of a carbohydrate solution and placebo solution to ingest and a carbohydrate mouth rinse and placebo mouth rinse. Further exploration is needed to examine the effects of sports performance mouth rinsing through the means of a performance

test that incorporates perceptual, metabolic and endocrinal measures in order to better understand the complex mechanisms associated with carbohydrate supplementation and its effect on endurance performance. Speculations that are currently publicised regarding the mechanisms behind sports performance with carbohydrate mouth rinsing and carbohydrate ingestion remain as speculations until there is an attempt made to analyse all of these aspects together.

Due to the equivocal research the purpose of the present study is to investigate whether there are individual and/or additive effects of carbohydrate mouth rinse, fluid intake and carbohydrate ingestion on 1-h time trial cycling performance. The project further investigates the response in circulating markers of fuel utilization.

2.1 Hypotheses:

H0: That there will be no difference in performance, perceptual, endocrinal or metabolic measures between the carbohydrate mouth rinse, the carbohydrate ingestion, placebo mouth rinse and placebo ingestion trials

H1: That ingesting a carbohydrate solution during high performance exercise will stimulate the effective use of metabolic fuels by elevating circulating glucose and insulin and this will result in an increase in endurance performance more so than mouth rinsing with a carbohydrate or placebo solution or placebo ingestion alone.

3.0 Literature Review

3.1 Introduction

The aim of the present literature review was to examine the effects of carbohydrate ingestion on exercise performance and to discuss the mechanisms regarding the efficacy of such results. A further aim was to examine the recent concept of sports performance mouth rinsing using a carbohydrate solution. This review will critically examine the underlying mechanisms for purported ergogenic effects seen in sports performance mouth rinsing.

Key limiting factors of endurance performance

Endurance exercise involves a complex integration of many physiological functions (Robergs, 1997). A key principle in exercise physiology is that work requires energy and as the duration of exercise increases there is a greater reliance on ATP resynthesis through oxidative phosphorylation to maintain cross bridge cycling. Consequently, the rate at which oxygen is used during prolonged submaximal exercise is a measure of the rate at which ATP is generated (Bassett et al, 2000). A higher maximal oxygen uptake ($\dot{V}O_2max$) is known to benefit performance in endurance events (Bassett, 1997), and key limiting factors of endurance performance have been associated with metabolic responses. During prolonged exercise there is evidence of decreased carbohydrate availability resulting in significant glycogen reductions and low blood glucose levels and, as a result of the above, free fatty acid concentrations are elevated (Wagenmakers et al, 1991). Changes of mood, motivation, processing of somatosensory information, perceived exertion and excitability of the motor cortex have also been proposed to alter CNS function resulting in a decrease in performance in both prolonged and high-intensity exercise (Nybo, 2003; Davis et al, 1997; Winnick et al, 2005). Issues relating to changes in mood, motivation and perceived exertion will be examined in more detail later.

Energy metabolism moderate to high intensity prolonged exercise

When discussing endurance performance and the aetiology of fatigue, we need to consider a number of metabolic factors, including aerobic energy metabolism. Athletes work at a high intensity during endurance events and the near-maximal efforts require a mix of anaerobic and aerobic energy metabolism (Duffield, 2004; Laursen, 2010). For an intense exercise event that lasts longer than 75 s, the total energy output is generally driven by the aerobic system (Laursen, 2010).

Substrate utilisation

The main determinants of substrate utilisation during exercise are the intensity and the duration of exercise as well as the pre-exercise carbohydrate stores and the training status of the athlete (Jeukendrup, 2010). Fat and carbohydrate are the two main substrates oxidized by skeletal muscle during exercise (Krogh, 1920). Endurance sports such as cycling are generally prolonged in activity and a typical road race or an off road cross-country event could range in duration from one to five hours (Faria, 2005). The prolonged activity of cycling has an average intensity of 75% $\dot{V}O_2$max (Jeukendrup, 1997) and this corresponds to 60% and 40% of carbohydrate (both glycogen and glucose) and fats towards metabolism respectively, thus showing the importance of carbohydrates in endurance exercise (Wagenmakers, 1989).

During moderate – high intensity exercise, the anaerobic metabolic pathways are utilised more (Wilmore, 2005). There are two types of anaerobic energy systems, firstly there is the high energy phosphates, ATP adenosine triphosphate and creatine phosphate (ATP/CP) which are stored in the muscle cells in limited amounts and secondly there is anaerobic glycolysis. In the absence of oxygen, anaerobic glycolysis uses glucose and glycogen as a fuel particularly when the rates for ATP are exceeded than what can be provided by aerobic metabolism. With the rapid breakdown of glucose there are increased rates of formation of lactic acid as a consequence (Tabata, 1996).

As emphasised above, during hard muscular work, energy production is mainly through carbohydrate consumption. Carbohydrate is usually stored as glycogen in the muscle and it is utilised for energy production (Bergstrom, 1967). When this glycogen store is reduced, glucose supplied by the blood is utilised more. During prolonged exercise, muscle glycogen has been shown to decrease (Bergstrom, 1967). Epinephrine is released via the adrenal glands as a direct effect of exercise. Simply stated, the greater the exercise intensity, the greater the epinephrine release (Farrell, 1986). Once the epinephrine is released it stimulates the breakdown of muscle glycogen and this then makes glucose available for the working muscle cells (Farrell, 1986). Epinephrine also promotes the breakdown of glycogen to glucose in the liver where some of the glucose will circulate and provide support to working muscle (glycogenolysis). Cortisol is also released during prolonged exercise which also supports in the breakdown of muscle glycogen and in gluconeogenesis in the liver (Farrell, 1986).

The rate of glycogen utilisation is related to the relative workload rather than to the absolute workload. Muscle glycogen levels of the working muscle fall steadily during exercise and in this case there is an increased output of glucose from the liver and an increased utilisation of fat (Bergstrom, 1967). Muscle fibres and their glycogen content during different intensities and pedalling rates have been examined. Gollnick et al (1974) found that glycogen depletion in individual fibres during exercise may be the cause of muscle fatigue which results in a decrease in performance, even when the exercise was of a lower intensity (50-60% $\dot{V}O_2max$) but of a long duration.

Glucose availability is important during prolonged exercise and the related increase in peripheral glucose requirements during prolonged exercise has been highlighted above (Lieberman, 2002). The principle hormone responsible for control of glucose metabolism is insulin. Insulin is synthesised in the pancreatic β-cells of the islets of Langerhans as the precursor proinsulin, which is then cleaved to form one molecule of insulin and one molecule of C-peptide. C-peptide is then released into the circulation at concentrations equimolar to those of insulin and is extracted by the liver in very small amounts (Coggan, 1995).

The plasma glucose concentration controls the secretion of insulin (Mari, 2001). Insulin's main function is to control the uptake and utilisation of glucose by the glucose transporter in peripheral tissues and also aids in the inhibition of gluconeogenesis and glycogenolysis (Horowitz, 1999). Gluconeogenesis is the synthesis of glucose from non-carbohydrate precursors such as glycerol, ketoacids or amino acids and lactate (Faria, 2005). During exercise, insulin activity decreases and becomes more efficient (Faria, 2005). Exercise activates non-insulin mediated glucose transport pathways and, when insulin concentrations decrease relative to the counter-regulatory hormones (glucagon, epinephrine, norepinephrine, growth hormone and cortisol), the liver is stimulated to release stored glucose. During prolonged exercise (> 60 min) blood glucose that is transported into cells is replaced by glucose from hepatic stores and concentrations of plasma free fatty acid (FFA) increases (Horowitz, 1999). Blood-borne FFA stores become increasingly important as the carbohydrate stores become depleted (Bangsbo, 1994).

Fatty acids are stored in the form of triacylglycerols within adipocytes of adipose tissue and can be mobilized for use by peripheral tissues as a response to the energy demands (as reported in Stevenson, 2009). The process of fatty acid oxidation (β-oxidation) occurs in the mitochondria. However, fatty acids must be activated in the cytoplasm before being oxidized in the mitochondria (as reported in Stevenson, 2009. The result of this activation process is the consumption of two molar equivalents of ATP: Fatty acid + ATP + CoA → Acyl-CoA + PP_i + AMP. Each round of β-oxidation produces one mole of NADH, one mole of $FADH_2$ and one mole of acetyl-CoA. The acetyl-CoA then enters the tricarboxylic acid (TCA) cycle, where it is then oxidized to CO_2 along with NADH, $FADH_2$ and ATP. The NADH and $FADH_2$ can then enter the respiratory pathway for the production of ATP (Stevenson, 2009). During prolonged moderate intensity exercise, as the carbohydrate fuels deplete, there is an increase in fat oxidation. This is the result of increased levels of blood lipase during low-moderate prolonged exercise.

3.2 Fatigue

Muscle fatigue – a complex subject, has been defined as 'the failure to maintain a required or expected power output (Edwards, 1983) or 'a decreased force generating capacity'(Vollestad

et al, 1988). There are many potential reasons for fatigue during endurance exercise; lack of fuel is one of these aspects.

Substrate depletion

Fatigue is thought to be due to a depletion of glycogen whereby the body cannot carry on at a given work rate due to a lack of substrate (Costill et al, 1992). In reality, many changes occur within and around muscle cells during intense exercise that can lead to fatigue, with substrate depletion being only one (Tabata et al, 1996).When exercising at intensities of 60-85% $\dot{V}O_2$max, fatigue occurs alongside with the depletion of muscle glycogen (Sahlin et al, 1998) and the result of this is displayed as a reduction of both high intensity short and prolonged exercise performance (Bergstrom et al, 1967) both of which may influence endurance performance.

Although the exact mechanism of glycogen depletion causing fatigue is not fully known it is likely due to interacting factors associated with the metabolic processes of muscle contraction (Costill et al, 1977). Fatigue may be due to the imbalance of ATP formation and ATP consumption which results in an energy deficiency and impaired force generation (Bigland-Ritchie, 1987). As explained earlier, during prolonged exercise, muscle glycogen concentrations decrease and this may result in an impairment of the contractile process (Spencer et al, 1991). Faria et al (2005) also suggests that prolonged exercise impairs muscle strength capacity associated with changes in contractile and neural properties of the leg extensors. There appears to be no relationship between fibre type and subsequent reduction of strength during cycling exercise and that in fact a central component of fatigue exists (Faria et al, 2005).

Central factors of fatigue

Central fatigue has been defined as a ‘negative influence that exists despite an individual’s full motivation’ (Davis, 1997). During fatiguing exercise there have been recorded changes in central enkephalinergic, dopaminergic and serotoninergic systems (Bailey, 1993; Persson, 1993). Increased concentrations of serotonin and the neurotransmitter 5-hydroxytryptamine

(5-HT), reduced concentrations of dopamine and also the accumulation of ammonia later in exercise have been linked with an impaired CNS function and are believed to be related to higher FFA concentrations in the blood (Lieberman et al, 2002; Winnick et al, 2005). These systems, and their associated hormones, have been postulated to control motivation, pain, vigilance, and tolerance (Gandevia, 1998; Winnick et al, 2005). They have also been found in the involvement of feelings of tiredness, lethargy and also in the perception of pain (Newsholme et al, 1987). Other neuroendocrine changes can alter the availability of substrates for muscle contraction and manipulation of these systems could in fact alter these central effects of fatigue (Pottier et al 2008; Gandevia, 1998).

The discomfort and pain associated with prolonged exercise that an athlete experiences will lead to decreases in CNS function. During prolonged exercise afferent information from muscles, joints, lungs and core temperature receptors is being conveyed and this may be perceived as 'unpleasant' and may consciously or unconsciously lead to an inhibition of motor output known as 'central fatigue' (Chambers, 2009). It is still unclear which pathways are involved in the motor output inhibition during exercise (Chambers et al, 2009). During periods of long duration time trial exercise, individuals must select a self-paced work rate, which will delay fatigue and help optimise performance. This is known as pacing strategy and is thought be a function of biochemical and metabolic afferents to the CNS which are developed from past experiences (Johnson et al 2006; St Claire Gibson et al 2001). A number of theories exist as to what constitutes 'central fatigue' and as a result, further research in the area is needed.

Temperature regulation

During periods of high work rates metabolic heat production can exceed 80 $kJ \cdot min^{-1}$ (Maughan et al, 1991) and eventuate in rises in core temperature. Detection of increased heat stimulates heat receptors situated in the anterior hypothalamus and cutaneous blood flow is also increased in order to transport the heat from the core to the skin and sweating is also initiated (Gisolfi, 1992). Dehydration may then occur as a consequence and this may have an impact on performance as fluid in intracellular and extracellular compartments of the body is reduced.

Dehydration and exercise

Sweating is a physiological response to prolonged exercise and is required for the dispersion of the heat produced during energy metabolism. However, the secretion of sweat also represents the substantial loss of vital body fluids (water) and ion losses and this can impair endurance performance (Robergs et al, 1997). With the increase in sweat loss from exercise there is an increased K^+ efflux from the exercising muscle as well as an increase in intracellular water and together this results in a decrease of intracellular K^+ (McKenna, 1992). This ionic imbalance during intense exercise indicates that the ion fluxes exceed the capacity of the Na^+/ K^+ pump to maintain an ionic balance; the result is a reduced membrane potential in the muscle which therefore leads to a decrease in muscle contractile performance (McKenna, 1992).

Losses of 2% body weight during exercise have been reported to decrease endurance performance by 10% (Saltin et al, 1988). Inadequate fluid balance throughout prolonged bouts of exercise can result in harmful physiological effects, such as increased heart rate and temperature (Hamilton et al, 1991; Hargreaves et al, 1996). Moreover, as sweat is hypotonic, the intracellular water tends to become hypertonic which therefore contributes to intracellular dehydration (Saltin et al, 1988). Fitts et al (2004) suggested that rising core temperature may cause fatigue in the muscles through impairing mitochondrial respiration and reduced central drive. As a result of the increase in heart rate, dehydration occurs due to a decrease in stroke volume from the lower volume of blood plasma, which is a large proportion of water (90%). During severe dehydration and with an increase in viscosity of blood, it is also possible for heart rate values to approach maximal levels even though the exercise may be of a submaximal nature (Robergs et al, 1997). Dehydration has also been implicated in having a negative influence on mental functioning (Winnick et al, 2005).

Fatigue is a multidimensional phenomenon and depends on the exercise itself, the status of the individual in terms of training and their physiology and also the conditions of the environment (Brooks et al, 1985). Fatigue may involve a depletion of energy supplies or an

accumulation of waste products, changes in pH, depletion of nervous system transmitters and dehydration (Kraemer, 1983).

3.3 Performance

Hydration

Athletes should take adequate care with hydration both before and during endurance exercise to counteract dehydration (Saltin et al 1988). At present, endurance exercise athletes and athletes of different backgrounds use many different products for hydrating including solutions of water, carbohydrates, electrolytes and glycerol.

Carbohydrate ingestion and performance

The information displayed in Table 3.1 is a brief summary of papers that have examined carbohydrate ingestion and exercise performance. Previous studies have shown that ingestion of carbohydrates during prolonged endurance exercise, intermittent and high-intensity exercise can improve performance (e.g. Lieberman et al, 2002; Coggan et al, 1988; McConell et al, 1999). McConnell et al (1999) suggested that the improvement in performance was due to an enhanced ability to resynthesize ATP at a given intensity of exercise for a prolonged duration. Other potential mechanisms include better maintenance of blood glucose and carbohydrate oxidation when carbohydrate is ingested (Coggan et al, 1988; Coyle et al, 1992; Mitchell et al, 1989); However, others believe that it could be due to more efficient sparing of muscle glycogen, delaying glycogen depletion (McConell et al, 1999; Nicholas et al, 2000; Rollo et al, 2010; Winnick et al, 2005). The reasons for this discrepancy could relate to methodological considerations during intermittent high-intensity exercise with team based players (Micholas et al, 2000; Winnick et al, 2005) or running (Rollo et al, 2010) or cycling based exercise time trial protocols (Jeukendrup et al, 2008) with triathletes. Due to these discrepancies in understanding the mechanisms behind the ergogenic effects seen, further research is needed.

Table 3.1: A brief summary of some studies investigating carbohydrate ingestion on exercise performance and endurance capacity. (Carbohydrate = CHO; Placebo = PLA)

Reference	Experimental conditions	Performance measurement	Performance Effect
Coggan et al (1988)	6% CHO vs. PLA	Time to exhaustion with 15 min bouts of 60-85% $\dot{V}O_2max$	Maintained higher intensities and delayed fatigue by 30 min in CHO compared to PLA
Anantaraman et al (1995)	10% CHO vs. PLA, 300 ml every 15 min exercise, and pre exercise	60 min intense cycling time trial	11% faster with CHO
Below et al (1995)	6% CHO vs. PLA 4 $ml \cdot kg^{-1}$ every 10 min	50 min at 80%$\dot{V}O2max$ and 10 min time trial	6% faster in CHO
Tsintzas et al (1995)	6% CHO vs. PLA	Running at 70% $\dot{V}O_2max$ until exhaustion	Reduction in muscle glycogen in type I muscle fibres after 60 min with CHO
Jeukendrup et al (1997)	7.6% CHO vs. PLA	60 min cycling time trial	2.6% faster in CHO trial
McConell et al (1999)	CHO vs. PLA	Endurance running time trial	No difference in performance, lack of statistical power
McConell et al (2000)	6% CHO vs. PLA	Time to exhaustion at 83% $\dot{V}O_2max$ at 31°C	12.4% increase in time to exhaustion with CHO
Lieberman et al (2002)	6% CHO vs. 12% CHO vs. PLA	Performed a range of physically demanding tasks	Both CHO groups performed the exercise tasks faster than PLA
Carter et al (2004)	Infused 20% glucose in saline vs. not infused (PLA)	Cycling 40km time trial	No effect on performance
Desbrow et al (2004)	6% CHO vs. PLA	60 min time trial	No effect
Carter et al (2005)	6.4% CHO vs. PLA	Time to exhaustion at 73% $\dot{V}O2max$	13.5 % increase in time to exhaustion in CHO
Winnick et al (2005)	6% CHO vs. PLA 3 $ml \cdot kg^{-1}$	60 min intermittent shuttle running + Stroop colour test, Profile of Mood States	Increase in performance and enhancement of motor skills
Jeukendrup et al (2008)	6% CHO vs. PLA	16km time trial (25 min)	No effect
Nybo et al (2009)	10% CHO solution vs. PLA 200 ml every 15 min	8 weeks exercise training	Influence on muscle training adaptations but no increase on fitness

Endurance capacity

There are two types of endurance tests, those that measure endurance capacity and those that measure endurance performance. Much of the research investigating the influence of carbohydrate ingestion on exercise has examined the effects on endurance capacity (exercise to volitional fatigue), (Bishop et al, 2002; Coyle et al 1992). A study conducted by Carter et al (2003), consisting of cycling to exhaustion at 73% $\dot{V}O_2$max in 35°C, found a 14% increase in time to fatigue with carbohydrate ingestion when compared to a placebo. Blood glucose was the only metabolic parameter examined and in this protocol there was no attempt to analyse participant perception even though they were exercising in a temperature much higher than what they would normally cycle in (Carter et al, 2003). Carter et al (2003) suggested that the onset of fatigue was delayed through improved maintenance of glycogen and this related to an ability to limit lactate accumulation at higher temperatures.

A study where participants underwent a running protocol at 70% of $\dot{V}O_2$max until exhaustion, found that there was a reduction in muscle glycogen breakdown in type I muscle fibres after 60 min with the ingestion of a carbohydrate solution (Tsintzas et al, 1995). The type II muscle fibres seemed unaffected with carbohydrate ingestion. The majority of evidence suggests that with carbohydrate ingestion during endurance capacity based tests, performance is improved through maintenance of euglycemia and higher rates of carbohydrate oxidation (Jeukendrup, 2004). Research suggests that endurance capacity is less reliable than endurance performance as endurance athletes tend not to exercise to exhaustion during sports events (Hopkins et al, 2001).

Endurance performance

Hopkins et al (2001) suggests that performance tests are more sensitive and are a better representation of 'real life' sporting events than endurance capacity tests and they also report a number of measures associated with performance (Rollo et al 2010). Up until recently, the positive effects seen with carbohydrate feeding were only demonstrated with exercise lasting over two hours in duration (Jeukendrup et al, 2008). As explained earlier, carbohydrate ingestion during prolonged exercise has been found to maintain high rates of carbohydrate oxidation (Jeukendrup, 2010). Although there is a large amount of research examining the

influence of carbohydrate ingestion on endurance capacity, there are fewer studies examining the influence on endurance performance and thus recently, endurance performance based tests have been the centre of interest.

Jeukendrup et al (1997) conducted a study where cyclists performed a 40-km time trial with and without ingestion of a carbohydrate-electrolyte drink. The authors found that participants in the carbohydrate-electrolyte ingestion group performed 2.3% (~1 min) faster than the placebo group. However, a marginal difference such as a 2.3% improvement in performance may be meaningful in a race situation. Although the improvement in performance time was statistically significant the actual percentage of improvement was marginal when compared to the placebo solution. Other studies have found similar results with improvements in performance with shorter duration of exercise. An earlier study by Anataraman et al (1995) investigated the effects of carbohydrate ingestion during 1 h of moderate to high intensity cycling where subjects had to perform as much work as possible. The authors found that in the carbohydrate ingestion trial performance was improved by 11% compared with the placebo trial. However, the improvement that was reported was not statistically significant and was possibly due to the small sample size (n=5).

Training with carbohydrates

Nybo et al (2009) conducted a study with physically inactive men to investigate how chronic training responses are affected by glucose supplementation versus a placebo over 8 weeks. The authors concluded that training with carbohydrate supplementation influenced various muscular training adaptations however improvements in cardiorespiratory fitness and reductions in fat mass were not affected in the carbohydrate ingestion trial. The placebo group in this study was reported to have a higher initial fat mass prior to the aerobic training when compared with the ingestion trial. The authors explain that the greater change observed in the placebo group's performance results may be because the participants had a greater potential to improve with the training as they started the protocol with an inferior level of fitness and body mass; hence the improvements would have been experienced to a greater magnitude than the carbohydrate group. Studies have also analysed endurance performance in different temperatures, and found that cyclists improved time trial performance in 31°C

heat by 6% when carbohydrate was ingested when compared to water alone (Below et al, 1995). The authors reported that there were no significant differences in esophageal temperature between carbohydrate and placebo groups despite the significant difference in performance with carbohydrate ingestion and blood glucose was the only metabolic parameter analysed in this study. There has been conflicting research on the performance benefits with carbohydrate ingestion as seen above. Some of the research has not observed a difference in sports performance when carbohydrate was ingested (Desbrow et al,2004; McConnell et al, 2000; Nikolopoulos et al, 2004). Many of these studies did show a trend for performance benefits with carbohydrate ingestion; therefore there may have been issues with statistical power.

Cognitive performance

Ingestion of carbohydrates during exercise has also shown improvements in performance by delaying impaired CNS function that generally occurs towards the end of exercise (Nybo et al, 2003; Winnick et al, 2005). Nybo et al (2003) proposed that it is possible to impair voluntary performance during exercise by an altered CNS function. With an altered CNS function mood, motivation, perceived exertion, excitability of the motor cortex and processing of incoming somatosensory information can be affected and thus impairing performance in this manner (Davis et al, 1997; Winnick et al, 2005). Although there is plentiful research displaying these effects of CNS function, the exact mechanisms that explain these changes are yet to be determined. Winnick et al (2005) investigated the effects of ingesting a 6% carbohydrate-electrolyte solution on physical and CNS function during an intermittent high-intensity protocol. Their results showed a performance enhancement in whole body motor skills and physically orientated tasks (including sprinting and jumping) with CHO ingestion. Lieberman et al (2002) also found improvements in performance when carbohydrate ingestion was investigated during exercise in the fed state. Through the use of a profile of moods states (POMS) questionnaire, the authors reported that in the 12% carbohydrate ingestion trial there were higher vigilance, higher vigour and less confusion than in the placebo trial and the 6% carbohydrate trial and that further research is needed to understand the effects of carbohydrate supplementation on anaerobic exercise (Lieberman et al, 2002).

Carbohydrate ingestion – mechanisms of action

The effects of carbohydrate ingestion during low and moderate intensity exercise may be related to insulin response differences (Horowitz et al 1999). In low intensity exercise plasma insulin levels increases two to three times above fasting levels with carbohydrate ingestion and skeletal muscle glucose uptake increases (McConell et al, 1999). The increase in plasma insulin levels causes a decrease in plasma FFA concentration resulting in a suppression of triglyceride hydrolysis (lipolysis). The end result is an increase in carbohydrate oxidation and a decrease in fat oxidation during exercise (McConell et al, 1999). Horowitz et al (1999) and other authors explain that when lipolysis was suppressed to reduce fat oxidation, muscle glycogen oxidation increased so that energy during exercise was maintained (Coggan et al, 1991).

During moderate intensity exercise with a carbohydrate supplement, it has been postulated that the insulin response is suppressed and there is no reduction in fat oxidation even though there are suppressions in plasma FFA and glycerol concentrations (Coyle et al, 1986; Hargreaves et al, 1988). This is due to the human body having a relatively small reservoir of unesterfied fatty acids (10–40 mmol/kg) where the rate of fat oxidation cannot exceed for more than a few minutes of exercise the rate of lipolysis (Hargreaves et al, 1988). A low lipolytic rate can then limit fat oxidation by reducing FFA amounts that are available for oxidation. In moderate intensity exercise when carbohydrate is ingested, extra energy is provided to maintain carbohydrate oxidation and thus an improvement with performance is seen (Horowitz et al, 1999). The exact mechanism that reduces fat oxidation in the exercising muscle is still unknown. Jeukendrup et al (1997) estimated that only 5-15g of exogenous carbohydrate is oxidised in the first hour of exercise and with such a small contribution of the exogenous carbohydrate to the overall oxidation rate there may be a non-metabolic explanation for the improved moderate-high intensity exercise (60-80% $\dot{V}O_2max$) performance when carbohydrate is ingested.

The amount of time exercising also plays a significant role in determining performance improvements with carbohydrate ingestion. Indeed exercise shorter than 30 min in duration has not been shown to benefit from carbohydrate ingestion (Jeukendrup et al, 2008; Palmer et

al, 1998). These findings may be attributed to insufficient time for the carbohydrates to absorb as the exercise was short in duration and high in intensity. Improvements that have been seen during exercise of shorter duration (<60 min) with higher intensities (80-85% $\dot{V}O_2$max) cannot be explained by the same mechanisms that have in the past explained improvements seen in performance with prolonged exercise protocols of >2 h exercise (Jeukendrup et al, 2008). In prolonged exercise the benefit of exogenous carbohydrates is related to the maintenance of euglycemia (normal levels of glucose in blood) late in exercise when the liver and muscle is depleted in glycogen stores. Pottier et al (2008) explains that the ergogenic effects of carbohydrate ingestion seen in exercise of shorter durations (<60 min) and of higher intensities (>75% $\dot{V}O_2$max) is much harder to explain as the human body contains enough glycogen for this type of work and hypoglycaemia is unlikely. The authors suggest the underlying mechanism may be a result of a higher glucose oxidation rate caused by carbohydrate consumption. However, there is conflicting evidence with this theory as other studies have shown no changes in carbohydrate oxidation between a carbohydrate ingestion trial and a placebo trial during the initial 45-50 min of steady-state exercise (75-80% $\dot{V}O_2$max; (Below et al 1995; Desbrow et al 2004; Pottier et al 2008). During higher intensity exercise, plasma glucose concentrations do not decrease and have been found in some cases to increase because of increased hepatic glucose output; in fact carbohydrate oxidation rates are high and are mainly resulting from muscle glycogen and therefore short duration, high intensity exercise is likely not influenced by ingestion of carbohydrates (Romijn et al, 1993).

In a study examining carbohydrate as a fuel, cyclists performed a 40-km time trial where either a glucose solution or a saline solution was infused (Carter et al, 2004a). In the trials where glucose was infused, the blood glucose concentrations and glucose disappearance were twice as high. With this in mind there was no effect on performance even though it was thought that glucose had been taken up and oxidised in the muscle (Jeukendrup et al, 1999). The presence of a central mechanism was then speculated in an attempt to explain the improvements in performance observed (Jeukendrup et al, 1997). Due to the discrepancy in the mechanisms surrounding carbohydrate ingestion and endurance performance further research is needed.

Carbohydrate ingestion and perception/mood

On the days preceding exercise it has been recognised that the capacity to sustain prolonged (2-3 h) moderate intensity (60-75% $\dot{V}O_2max$) exercise, increases with carbohydrate intake (Bergstrom et al, 1967). In one study, prior to a cycling time trial, a glycogen reducing exercise and two days of a low carbohydrate diet were administered to a group of endurance athletes (Johnson et al, 2006). The authors reported that there was an earlier onset of fatigue and that the carbohydrate diet did not influence initial self-selected work rate during the prolonged cycling exercise. It must also be noted that participants were verbally encouraged to perform their best during both of the experimental trials. This could potentially pose as a problem as their performance may have been influenced by the encouragement (Andreacci et al, 2002). There are reports that frequent verbal encouragement can significantly increase performance when compared to no verbal encouragement (Andreacci et al, 2002). In a 'real-life' time trial the encouragement that took place in the study of Johnson et al (2006) would not have occurred. The authors explain that elite competitive cyclists are characterised by high levels of motivation and that the likelihood of differences in the level of encouragement between treatments is low. However, there is evidence lacking in this particular area of research and therefore future research should attempt to control all factors that may have an external effect on performance during laboratory experiments.

In relation to carbohydrate ingestion and mood effects, Winnick et al (2005) found an enhanced external rating of the overall mood state late in exercise in the carbohydrate trial. These results however failed to show positive effects of carbohydrate ingestion on cognitive function or changes in mood. Backhouse et al (2005) found that when carbohydrate was ingested during prolonged cycling, throughout the exercise, pleasure-displeasure was enhanced and the effect of perceived exertion was limited. A later study by Backhouse et al (2007), examining the effect of carbohydrate ingestion on the dimensions of affect and perceived exertion during high intensity intermittent exercise, found that perceived activation was higher in the carbohydrate trial in the last 30 min when compared with a placebo solution. However, there were no further treatment effects noted with pleasure-displeasure. The authors suggested that further research is needed to explore the influence of the type and dose of carbohydrate used. They also suggested that there is indeed research lacking with the mechanisms surrounding perception and mood and blood glucose concentration (Backhouse et al, 2007).

Lieberman et al (2002) found that ingesting a carbohydrate solution during a day of sustained aerobic activity showed an improvement in vigilance and increases in vigour and decreases in confusion when the Profile of Mood States (POMS) questionnaire was used. However, due to the lack of research surrounding the effects of carbohydrate ingestion on mood, the authors explained that further research is needed in order to support their findings. Later studies found that carbohydrate supplementation during exercise reduced the exercise stress, as indicated by lower levels of circulating adrenaline and cortisol concentrations (Febbraio et al, 2002; Nybo et al, 2009). These studies suggest that there may be a 'central effect' with carbohydrate and the human body's exercise performance and this effect on the CNS can influence an athlete's perception, emotions and motivation in relation to performance.

Negative aspects of carbohydrate ingestion

Although there are many studies that show the benefits of carbohydrate ingestion on exercise performance (e.g. Coggan et al, 1988; Davis et al 1997; McConell et al 1999; Below et al, 1995; Lieberman et al 2002; Jeukendrup et al, 2004; Jeukendrup et al, 2010) there is also evidence to suggest there may be potential ergolytic effects, including bowel discomfort and gastrointensitnal (GI) distress (van Nieuwenhoven et al, 2005). Complaints involving the GI tract range from reflux, nausea, upper abdominal cramping, bloating in the upper GI tract (stomach and oesophagus), urges to defecate, increased frequency of bowel movements, flatulence and effects of diarrhoea in the lower GI tract (small bowel and colon) (van Nieuwenhoven et al, 2005). The discomfort experienced may have a negative impact on an athlete's performance. GI discomfort is common among many athletes, and in particular runners, where it is thought the 'up and down' motions of running can put unwanted stress on the abdominal organs (Rehrer et al, 1991).

Rehrer et al (1991) investigated the prevalence of GI discomfort and dietary intake on triathletes. The authors found that those who suffered more from GI complaints had ingested hypertonic carbohydrate solutions during exercise and that others consumed food high in dietary fibre, fat or protein before competition. Nieuwenhoven et al (2005) compared the effects of ingesting three different sports drinks, including a carbohydrate-electrolyte solution, a carbohydrate-electrolyte solution containing caffeine and a control solution of

carbonated mineral water, on cycling performance and GI complaints. The authors found that the drinks containing carbohydrate had more GI complaints compared to water. Sports drinks are thought to empty from the stomach quickly (Rehrer et al, 1991) and contain less than 8% carbohydrate. Higher amounts of carbohydrate solutions are known to slow the gastric emptying rate resulting in less fluid becoming available for the body to use (van Nieuwenhoven et al 2005). Van Nieuwenhoven et al (2005) hypothesised that the composition of sports drinks may have not been optimal and the amount of carbohydrate taken up may have been too much. Athletes that regularly perform at their maximal capacities and ingest carbohydrate solutions, may lead to an even larger decrease in blood flow resulting in malabsorption of the carbohydrates ingested (van Nieuwenhoven et al, 2005). Osmotic difference could then develop causing an increase in intestinal water secretion instead of the preferred water absorption (van Nieuwenhoven et al 2005; Peters et al 2000).

Jeukendrup et al (2000) explains that the amount of ingested carbohydrate that is oxidised is termed 'oxidation efficiency'; high oxidation efficiency implies smaller amounts of carbohydrate remaining in the GI tract thus reducing the likelihood of developing GI disturbances. Multiple transportable carbohydrate sources have less carbohydrate remaining in the intestine and may result in less osmotic shifts and malabsorption (Jeukendrup et al, 2010). Therefore, carbohydrate solutions with both glucose and fructose may help decrease the risk of developing GI discomfort (Jeukendrup et al, 2008); however, more research is needed in this area to support these findings.

Taste transduction pathways - process of taste

When food or drink is placed in the mouth, taste receptor cells (TRCs) in the taste buds of the tongue are stimulated (Chandrashekar et al, 2006). There are five 'tastes' reported in previous research including sweet, salt, bitter, sour and umami (a savoury taste) (Chandrashekar et al, 2006). Upon arrival of food or drink in the oral cavity, electrical activity is transmitted to gustatory neurons (cranial nerves VII, IX and X) and these nerves innervate the taste buds which then relay the information to the primary taste cortex of the brain (Jeukendrup et al, 2010; Small et al, 2001). Regions of the brain that are reported to be activated by carbohydrate in the oral cavity may then provide an explanation for the ergogenic effects of a carbohydrate mouth rinse on exercise performance (Jeukendrup et al, 2010).

Mouth Rinsing with Carbohydrate

As emphasised above the benefits of ingesting carbohydrates on endurance performance is well established. Fatigue during prolonged exercise has been closely associated with muscle glycogen store depletion (Coyle, 1992). Much of the research discussed earlier in this literature review has investigated the effect of carbohydrate ingestion on endurance capacity and has shown that the time to fatigue often increases with the ingestion of a carbohydrate solution. As a result there have been theories of euglycemia maintenance and delaying muscle glycogen depletion late in exercise (Coggan et al, 1991; Coyle, 1992). Many of the endurance tests used in the past undergo a duration of 45-60 min and it has been postulated that during exercise of this duration endogenous glycogen stores are sufficient for the task at hand and that the provision of exogenous carbohydrate contributes minimally to carbohydrate oxidation of endogenous muscle glycogen. Thus suggesting there may indeed be a central effect associated with carbohydrate supplementation. There have also been links made between the transit time of the solution in the mouth and increases in endurance performance (Carter et al 2004a; Chambers et al 2009; Pottier et al 2008).

Possible mechanisms for ergogenic benefits of mouth rinsing

There are increasing speculations of non-metabolic explanations for the improvements observed in endurance performance with carbohydrate supplementation. Carter et al (2004b) further investigated these postulated non-metabolic effects. During a 60 min cycling time trial the influence of a carbohydrate mouth rinse vs. a placebo mouth rinse (not ingestion) on performance was analysed. As the solution was not swallowed, it was thought that the actions of carbohydrate consumption on the CNS could be assessed without measuring carbohydrate oxidation rates. Carter et al (2004b) found that there was a 2.8% increase in performance in the time trial when a carbohydrate mouth rinse was administered compared to a placebo mouth rinse. This lead to theories of possible stimulation of oral receptors that with a carbohydrate solution in the mouth activated reward/pleasure stimuli centres of the brain (Jeukendrup et al 2010). It has been hypothesised that a carbohydrate solution in the mouth may trigger oral receptors to activate the primary taste cortex and the putative secondary taste cortex in the orbitofrontal cortex (O'Doherty et al, 2001; de Araujo et al, 2003). The primary taste cortex and orbitofrontal cortex are believed to have projections to the dorsolateral prefrontal cortex, anterior cingulate cortex and ventral striatum – brain regions believed to mediate the behavioural and autonomic responses to rewarding/pleasure stimuli, including

taste (Rolls, 2007) such as glucose, salt (O'Doherty et al, 2001) and umami (de Araujo et al, 2003; Chambers et al, 2009). Rollo et al (2008) demonstrated that a carbohydrate mouth rinse increased total distance covered during a self-selected 30 min run when compared to a placebo mouth rinse and reported that the participants showed a trend towards higher perceived activation while mouth rinsing the carbohydrate solution, however, there was no statistical significance observed with the perceptual data in this study. There have also been speculations that carbohydrate stimulus in the oral cavity may counteract the negative afferent signals associated with central fatigue (St Claire Gibson et al, 2001).

Other than the potential of better understanding the mechanism of the effects of carbohydrate on high intensity exercise, there is also an idea relating back to those who suffer from GI discomfort during exercise when carbohydrate is ingested (Peters et al 2000; van Nieuwenhoven et al 2005; Rehrer et al 1991). A mouth rinse of a carbohydrate solution (instead of ingestion) has been postulated to decrease the risk of GI discomfort (Jeukendrup et al 2010). Due to the negligible amount of carbohydrate absorbed in mouth rinsing, there is less chance of GI discomfort which can influence performance (van Nieuwenhoven et al, 2005). Whitham et al (2007) found that use of a carbohydrate mouth rinse did not cause common GI discomfort experienced in runners and also suggested that the mouth rinse may in fact effect thirst sensation by reducing thirst, as the participants gargled the solution for 5 s which has been shown to temporarily reduce thirst in non-exercising but dehydrated participants (Seckl et al, 1986). Previous research has found that gargling with tap water can reduce thirst in dehydrated participants and that after eating, the human brain can sense a biochemical change and subsequently signals satiation (Liu et al, 2000). However, further research is needed in the analysis of thirst sensation and carbohydrate mouth rinsing to clarify this speculation (Whitham et al, 2007). Analysis of the temporal response after eating will be examined in more detail later on.

Mouth rinse and performance

There is increasing evidence suggesting that a carbohydrate mouth rinse solution can improve endurance performance; Table 3.2 shows a brief summary of papers that have examined the influence of mouth rinsing on various aspects of performance. Twelve endurance trained triathletes, underwent a 1-h cycling time trial with four different treatments, including a mouth rinse with a placebo solution, a mouth rinse with a carbohydrate electrolyte solution, a placebo solution to ingest and a carbohydrate-electrolyte solution to ingest; all solutions were

taste and colour matched (Pottier et al, 2008). The experiment aimed to directly compare the effects of rinsing the mouth with the effects of ingestion with a carbohydrate-electrolyte solution on high-intensity exercise performance; the same carbohydrate solution was used for both the ingestion trial and the mouth rinse trial. The authors found that participants improved their time trial performance by 2.5 min (3.7%) in the mouth rinse with a carbohydrate-electrolyte solution trial compared to an ingested carbohydrate-electrolyte solution and placebo solution. There was also a higher mean power output reported which was suggested to lead to a greater lactate concentration (+1.05 $mmol \cdot L^{-1}$ or +28.3%) as a consequence. It was suggested that the reduced oral transit time in the carbohydrate-electrolyte ingestion trial compared to the carbohydrate-electrolyte mouth rinse had less of an ergogenic effect on the results and the benefits observed on performance in the mouth rinse trial may suggest that there is a 'central effect'. The authors also speculated that fatigue signals may have been suppressed unconsciously by afferent carbohydrate signals from hypothesised carbohydrate receptors in the mouth. However, the trials were separated by approximately 48 h and the duration between the trials may be questioned as being too short that may have effected recovery time (Pottier et al, 2008). The results were weighted on performance with little emphasis on perceptual or psychological effects even though an earlier study by Carter et al (2004b) suggested that the improvements may be a result of a 'central effect'.

Self-selective exercise intensity has also been suggested as a possible reason for the difference between trials observed in an earlier study (Rollo et al, 2010). As the test required self-selected speeds, conscious alterations may have not been specific enough to detect any subconscious 'central effect' that has been hypothesised to play a major part in explaining the change in performance. Therefore, Rollo et al (2010) designed a study that investigated the influence of mouth rinsing a carbohydrate-electrolyte solution on 1-h running performance to examine whether mouth rinsing a carbohydrate-electrolyte solution altered blood glucose and plasma insulin concentrations at rest. In this study, 10 endurance trained male participants were asked to perform a 1-h running time trial; they were given either a 6.4% carbohydrate-electrolyte solution or a placebo solution to mouth rinse. They found that the carbohydrate-electrolyte mouth rinse was associated with a 1.5% (211 m) increase in distance covered compared to placebo but there were no changes in blood glucose during the exercise. The authors postulated that a longer transit time in the mouth may affect 'uptake' of glucose. They also suggested that their findings were a result of central effects and agreed with

previous research that carbohydrate mouth rinsing may indeed trigger reward centres in the brain thus supressing fatigue signals. Rollo et al (2010) also speculated that mouth rinsing

Table 3.2: A brief summary of studies investigating carbohydrate mouth rinsing on exercise performance and endurance capacity. (Carbohydrate = CHO, Placebo = PLA)

Reference	Trials	Performance measurement	Performance effect
Carter et al (2004)	6.4% CHO mr vs PLA mr	60min cycling time trial	2.9% faster with CHO mouth rinse
Whitham et al (2007)	6% CHO mr vs PLA mr	45 min treadmill run	No difference
Pottier et al (2008)	6% CHO mr vs 6% CHO ing	60 min cycling time trial intense	3.7% faster in mouth rinse
Rollo et al (2008)	6% CHO mr vs PLA mr 100 ml every 5 min	30 min treadmill run	Increase in self-selected running speed in first 5 min with CHO mr
Beelen et al (2009)	6.4% CHO mr vs PLA mr	60 min cycling time trial	No difference
Chambers et al (2009)	6.4% sweet CHO vs 6.4% nonsweet CHO 150 ml every 12.5% of time trial	60min cycling time trial	Both increase performance
Gant et al (2010) a	6.4% CHO ing vs PLA ing	30 min isometric elbow contraction	CHO ingestion improved performance
Gant et al (2010) b	6.4% CHO mr vs PLA mr	30 min isometric elbow contraction	CHO mouth rinse improved performance
Rollo et al (2010)	6.4% CHO mr vs PLA mr 25 ml every 15 min	60 min running time trial	1.4% faster with CHO mouth rinse
Rollo et al (2011)	6.4% CHO ing 2 ml·kg^{-1} vs 6.4% CHO mr 25 ml vs PLA ing every 15 min	60 min running time trial	2.2% faster with CHO ingestion

mr = mouth rinse; ing = ingestion

may be suitable for athletes who experience GI discomfort when ingesting carbohydrate drinks during exercise and that there may be no adverse effects and may even lead to an increase in performance. Chambers et al (2009) examined the effects of carbohydrate mouth

rinsing through magnetic resonance imaging (MRI) and found that both glucose and maltodextrin activated regions of brain (insula/frontal operculum, orbitofrontal cortex and striatum) associated with reward which are capable in mediating behavioural responses. However, in this study there was no reference made to recording the weight of the swill expectorate and therefore the responses observed may have been due to small amounts of the solution being swallowed.

Conflicting evidence with use of sports mouth rinsing

Not all research has shown benefits in performance with a carbohydrate mouth rinse. In the presence of a 6% maltodextrin mouth rinse there were no significant differences in distance covered or running speed in a 45-min running time trial test compared to a placebo mouth rinse (Whitham et al, 2007). There were also no differences in blood glucose or lactate concentrations or in ratings of perceived exertion between trials. The authors suggested that the 45-min time trial may not have been sensitive enough to detect any benefits of a carbohydrate mouth rinse. There were no other perceptual measures taken even though the authors speculated that mouth rinsing with carbohydrate may involve a 'central effect.' The participants also consciously altered their own speed during the time trial. Consciously altering speed is ecologically valid in conditions such as cycling where during competitions and events a cyclist changes gears regularly however, as the above research was a running protocol, the test may have not been a true representation of performance because the research was initially set out to examine the potentially unconscious 'central effects' that may occur with mouth rinsing a carbohydrate solution (Whitham et al, 2007). Also, the participants in this study were in a fed state because they were provided a standard meal before the test. This again could have had a detrimental effect on detecting the very small changes that are hypothesised to occur when a carbohydrate mouth rinse is being investigated (Rollo et al, 2011). The authors concluded that maintenance of energy supply of endogenous carbohydrate may have negated any potential central effects that may have occurred with a carbohydrate mouth rinse and as a result the blood glucose concentrations appeared to not decrease in the running time trial (Whitham et al 2007). The above protocol had only set out to test the effect of a carbohydrate mouth rinse versus a placebo mouth rinse on running performance; further clarity could have been made to understand this mechanism if both a carbohydrate ingestion trial and a placebo ingestion trial were included.

A study examining the effects of a 6.4% carbohydrate mouth rinse on a 1-h cycling time trial, found no differences in power output, heart rate or perceived exertion (Beelen et al, 2009). This study initially set out to further investigate the findings Carter et al (2004b) reported on the benefits on performance with a carbohydrate mouth rinse in the fasted state. They examined the effects of a carbohydrate mouth rinse in a more practical situation of testing i.e. a fed state. The protocol used was very similar to Carter et al (2004b) except a standardised breakfast was given to the participants 2 h prior to the test. The absence of an ergogenic benefit with a carbohydrate mouth rinse was attributed to endogenous glycogen stores being readily available after ingestion of the standardised breakfast to sustain the relatively short duration exercise (Beelen et al, 2009). In this experiment, blood glucose and lactate were not analysed and there was no attempt made to calculate the estimated amounts of endogenous and exogenous carbohydrate despite their conclusions. There were also no psychological measures taken even though the concept of mouth rinsing is based on central effects. It could be speculated that had the authors analysed subjective perceptions they may have had different findings.

Application of mouth rinsing

Although there is evidence suggesting there are benefits associated with mouth rinsing a carbohydrate solution on exercise performance, the practical application must also be addressed (Jeukendrup et al, 2010). Research has shown that mouth rinsing with a carbohydrate solution can in some cases improve endurance performance (Carter et al, 2004b; Chambers et al, 2009; Rollo et al, 2008). The research protocols have been based around the sports of cycling and running and there is research lacking in other areas of exercise including intermittent exercise as seen in games sports. Often intermittent games consist of either quarters or halves and the only time a player can ingest a carbohydrate solution is during these breaks. A sports performance mouth rinse may prove to be practical in this sense as it would be a more convenient method of utilising the potential ergogenic effects (i.e. invoking postulated ‘central effects’ through swilling rather than consuming much larger boluses). There are however, health and safety issues revolving around mouth rinsing as the athlete would be ‘spitting’ the solution out into the surrounding environment and issues relating to social acceptability may deter athletes from pursuing such a method. Due to the issues of hygiene and the practical aspects, further analysis is needed to examine if

sports performance mouth rinsing is possible in a 'real life' sporting situation. The role of dietary carbohydrates in weight loss has received considerable attention in recent years (Ma et al, 2005). Future research could examine the influence of a carbohydrate mouth rinse on its relation to weight loss (Atkins, 1998; Agatston, 2003).

Brain responses to mouth rinsing

Decreases that have been observed in perceptual scales such as the ratings of perceived exertion (RPE; Borg,1973) scale with carbohydrate ingestion during exercise may be explained through the maintenance of carbohydrate substrates. This corresponds with an improved maintenance of muscle contractile properties and neurological function or a central effect involving the stimulation of reward/pleasure stimuli in brain (Whitham et al, 2007). The beneficial effects have in the past been explained by stimulation of central nervous system function, increased cerebral glucose uptake and oxygen consumption and lower RPE towards the end of short intensive exercise (e.g. Backhouse et al, 2007; Nybo et al, 2003; Winnick et al, 2005). However, the postulated central effects of a carbohydrate mouth rinse may involve stimulation of cortical taste neurons (Carter et al, 2004b). Receptors in the mouth exist with neuronal communication to the nucleus accumbens (pleasure centre) in the brain (Jeukendrup et al, 2008). At present, evidence for this is lacking. Nevertheless, it is known that the brain can sense certain composition changes in the mouth and it has further been suggested that activation of these taste-related brain regions can influence emotion and behaviour (Kringelbach et al, 2004) and this may have an impact on exercise performance. The presence of polysaccharide receptors in humans is yet unknown (Carter et al, 2004b).

In an investigation using fMRI in the responses of the human brain to a carbohydrate mouth rinse during a cycling time trial, it was discovered that a sweet (glucose) and also a non-sweet (maltodextrin) solution activated the anterior cingulate cortex and ventral striatum of the brain (Haase et al, 2009). These areas were also unresponsive to an artificial sweetener (Jeukendrup et al, 2008). Recently, other research into neuroimaging with a carbohydrate solution has shown that the solution activates other brain regions (anterior insula, striatum) compared to an artificial sweetened solution (Frank et al, 2008; Haase et al, 2009). This suggests that there may be taste transduction pathways that respond independently to carbohydrate sweetness (Jeukendrup et al, 2008). Specific taste receptors for polysaccharides have yet to be identified (Sclafani et al, 2004) and, as maltodextrin is tasteless, it is unknown

how a carbohydrate with maltodextrin could be detected in the mouth, however, sweet mouth rinse during exercise of 1 h may affect pleasure reward stimuli as sweet has been associated with reward/pleasure in the past (Small, 2001). More research is needed in this area to clarify these assertions.

Liu et al (2000) examined the temporal response of the brain after eating with fMRI. Participants underwent a continuous functional scan for 48 min and ingested glucose at the 10 min point. The authors found two peaks in brain activity as a response of the glucose ingested – the first immediately followed ingestion and secondly, 10.3 min later suggesting that the second response was related to plasma glucose changes (Liu et al, 2000). The authors explained that the observed results represent a dynamic interaction between the fMRI response, the biochemical signal and plasma insulin. This was shown through an enhanced baseline activity of the hypothalamus prior to ingestion which was then decreased upon ingestion. The hypothalamus is known to regulate plasma glucose concentration through modulating insulin secretion (Liu et al, 2000).

A recent study using fMRI found that a non-sweet carbohydrate in the mouth produced a similar CNS response to that was obtained with glucose, suggesting that there may be a class of so far unidentified oral receptors that respond to the caloric property of carbohydrate independently of those for sweetness (Chambers et al, 2009). In the same experiment it was noted that after oral exposure to glucose, there was an increase in performance in the 1 h cycling time trial, with a 2% reduction in completion time in the glucose mouth rinse trial and a 3.1% reduction in the non-sweet maltodextrin trial compared to a placebo solution rinse. The fMRI results showed that there was in fact activation of the anterior cingulate cortex and the right caudate that forms part of the striatum when both a sweet (glucose) and non-sweet (maltodextrin) carbohydrate mouth rinse was used. In simpler terms, the improvement observed in exercise performance upon the presence of carbohydrate in the mouth may be due to the activation of certain regions of the brain which are thought to be involved in reward and motor control (Chambers et al, 2009). These regions, with emphasis on the dopaminergic pathways within the striatum of the brain, are thought to mediate emotional and behavioural responses to rewarding food stimuli (Rolls, 2007; Chambers et al, 2009). The dopaminergic system of the striatum has been implicated in arousal, motivation, and control of motor behaviour and further analysis in this area, with use of fMRI, may help explain the mechanisms surrounding carbohydrate mouth rinsing and the associated ergogenic effects on performance (Berridge et al, 1998).

To better understand the mechanisms seen with a carbohydrate mouth rinse on exercise performance and the associated activation of neural pathways, Gant et al (2010) investigated whether the presence of carbohydrate in the mouth can modify corticomotor excitability and voluntary force production. It was thought that if receptors in the mouth can activate neural pathways by the mere presence of carbohydrate that ingesting carbohydrate may have immediate neural consequences before the uptake of such carbohydrates into body tissues. After performing a fatiguing isometric elbow flexion exercise for 30 min, Gant et al (2010) found that normalised biceps brachii motor evoked potential (MEPs) in the participants was significantly greater immediately after ingestion of a carbohydrate drink compared to the placebo solution trial. The results in this study indicate that carbohydrate ingestion immediately facilitates the corticomotor pathway and thus increases voluntary force production (Gant et al, 2010) – a feed-forward mechanism originating from the mouth. The authors also conducted a second experiment investigating whether the presence of a carbohydrate mouth rinse facilitated significantly larger MEPs in the biceps brachii muscle. The presence of carbohydrate in the mouth and how it facilitates corticomotor output to 'fresh, un-fatigued muscle' was examined in more detail (Gant et al, 2010). The authors reported that there was an immediate ergogenic effect and that may indeed indicate that there is a neural mechanism. There were suggestions that chemoreceptors in the mouth, that upon taste stimulation, relay information regarding perception and energy density to the brain which then generates afferents that are known to change motor output (Gant et al, 2010). These findings were seen in both fatigued and 'fresh' muscle and were not affected by plasma glucose levels or the amount of fatigue (Gant et al, 2010).

Mouth rinsing vs. ingestion

A recent study examined the effects of mouth rinsing and ingesting a carbohydrate-electrolyte solution on 1 h running performance (Rollo et al 2011). After a 14-15 hour fast, 10 male recreational runners were given either a placebo drink to ingest, a 6.4% carbohydrate-electrolyte mouth rinse, or a 6.4% carbohydrate-electrolyte drink which they had to rinse in their mouth for 5 s prior to swallowing. Fingertip blood glucose and lactate samples were taken at 15-min intervals during the test and various perceptual constructs were also assessed. It was reported that ingestion of the carbohydrate-electrolyte solution significantly improved performance (by 2.2%) when compared to the mouth rinse and placebo. These results are in

contrast with the findings of Pottier et al (2008), who reported an enhanced performance (3.7%) with carbohydrate mouth rinsing relative to carbohydrate ingestion. The increases in running performance that occur, is unlikely a consequence of the increased rate of exogenous carbohydrate oxidation or elevated respiratory exchange ratio (RER) (McConell et al, 2000). McConell et al (2000) reported that the increased rate of carbohydrate oxidation is in fact a consequence of increased running speed with an increase in intensity during the carbohydrate-electrolyte ingestion trial. Again, underlying clear metabolic mechanisms were lacking in this study. There were speculations that carbohydrate ingestion does indeed have a positive effect on the CNS as seen in other studies using fMRI (Gant et al, 2010; Chambers et al, 2009). A placebo mouth rinse was not present in this study and there were limited psychological measures analysed despite the hypothesised central effects associated with carbohydrate supplementation. There were also limited metabolic measures analysed which suggests that more research is needed in order to clarify the relative contribution of a 'central' and/or 'peripheral' effect on exercise performance when ingesting a carbohydrate-electrolyte solution (Rollo et al, 2011).

Summary

The complex model of fatigue has been associated with limiting exercise performance. Fatigue may involve a depletion of energy supplies or an accumulation of waste products, changes in pH, depletion of nervous system transmitters and dehydration (Brooks, 1985; Kraemer, 1983). Although the underlying mechanisms surrounding fatigue are still unclear glycogen depletion has been associated with the metabolic processes of muscle contraction (Costill et al, 1977).

Recently, the benefits of carbohydrate ingestion and mouth rinsing on prolonged and short, intense exercise endurance performance has been noted (e.g. Carter et al, 2004b; Coggan et al, 1988; Gant et al, 2010). However, under these circumstances it is still unclear what the underlying mechanisms are when mouth rinsing with carbohydrate solution during exercise. Research suggests that blood glucose concentrations are maintained during this period and in some cases increase because of hepatic glucose output increases (Romijn et al, 1993). With a clear metabolic explanation not in sight, speculation of a 'central effect' of the CNS has been postulated to explain these improvements (Chambers et al, 2009; Gant et al, 2010;

Jeukendrup et al, 2010). Therefore, various groups (Pottier et al 2008; Whitham et al 2007; Beelen et al 2009; Rollo et al 2011) have examined the effect of carbohydrate mouth rinsing on endurance performance in order to better understand the issues surrounding the complex area of central vs. peripheral effects associated with carbohydrate supplementation on exercise performance.

With equivocal evidence regarding the ergogenic potential of carbohydrate mouth rinse and/or carbohydrate ingestion, further research is needed to understand any relationships between these concepts and the potential mechanisms involved. The beneficial effect of a carbohydrate mouth rinse on high-intensity exercise performance requires an increased availability of metabolic fuels either released from the liver or made available locally in the exercising muscle tissue. Such systemic effects are likely to reflect changes in metabolic signals or endocrine regulation of intermediary metabolism. However, the effects of a carbohydrate-electrolyte mouth rinse during or after high-intensity exercise on detailed perceptual, metabolic and endocrine responses has not yet been performed and the mechanism responsible for the improvements of high intensity performance remains elusive. (Carter et al, 2004b).

4.0 Methods

4.1 Subject recruitment

Ten recreationally trained cyclists and triathletes volunteered to participate in this study; however, due to unforeseen circumstances data from only 8 participants were reported. Participants were recruited by word of mouth and by posters and information sheets that were put up in local gyms, cycling shops and other universities in the Auckland area (Appendix 1). All procedures had prior approval by Massey University Human Ethics Committee (MUHEC). Before obtaining written consent (Appendix 2), the individuals who expressed an interest in the present study were verbally informed about the aims, procedures and the demands that the study would place upon them as well as informed on the possible risks and discomforts that may arise during the study. Participants were required to complete a medical history questionnaire and were fully aware of their right to withdraw from the study at any point (Appendix 3). The participants' personal training time ranged from 5 to 20 h per week, interspersed with competitive events.

4.2 Overall design

A randomised, double-blind, counterbalanced (subjects act as own control) trial was conducted through a latin square design. After an initial preliminary session, subjects were asked to record dietary intake over the 48-h period prior to each main trial. Each main trial took place over two days (Figure 4.1). On the evening of Day 1 the participants underwent a glycogen reducing exercise protocol followed by a low carbohydrate meal (~1 $g \cdot kg^{-1}$ body mass) and then a subsequent overnight fast (10-12 h). The following morning a performance time trial ride was conducted.

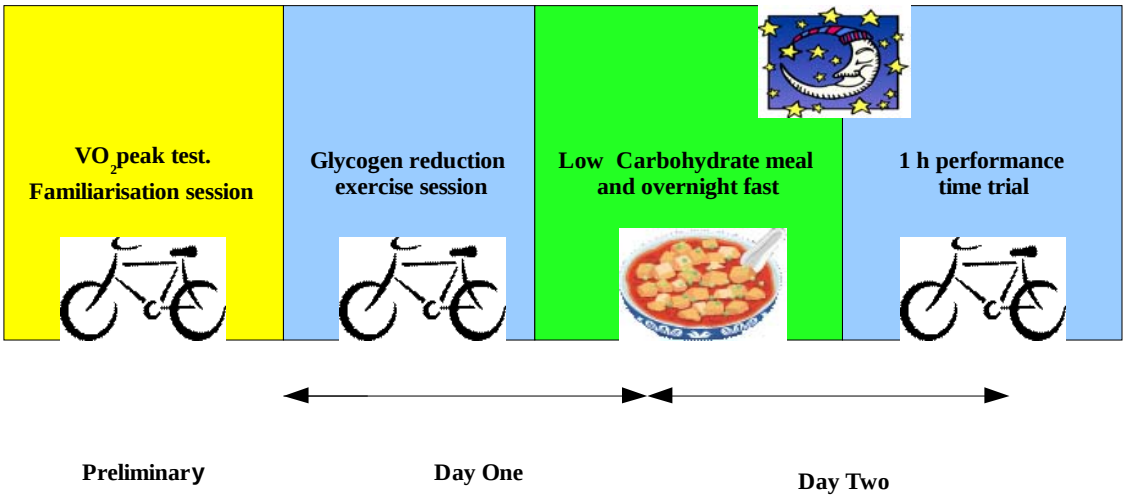

Figure 4.1: Overview of the experimental protocol

4.3 Physiological measures

4.3.1 Measurement of height and body mass

Nude body mass was measured using electronic scales (AND HV-200KGL, Australia) pre and post glycogen reduction exercise and pre and post the performance time trials. Height was determined using a stadiometer. The investigator ensured that subjects' heels were in contact with the heel board and an upright posture was assumed before any measurements were taken.

4.3.2 Measurement of expired air

Oxygen uptake ($\dot{V}O_2$) was measured using the Douglas bag technique (Douglas, 1911). The bags were the standard Siebe-Gorman pattern (fabric coated with vulcanized rubber), with total capacities ranging from 60 to 200 L. Participants wore a mouth piece with a turbine attached to a collection tube and Douglas bag. Oxygen uptake ($\dot{V}O_2$) was sampled during the preliminary session and during the main trial time at 20%, 40%, 60% and 80% of exercise. Once the mouth piece was inserted appropriately and had been in the participant's mouth for 30 s (to clear dead space) a 60 s sample was taken. The Douglas bag was analysed using a CO_2 and O_2 analyser (Servomex 1440 Gas Analyser, Crowborough, England) for CO_2 and O_2 content. The sample was subsequently analysed for gas volume (Harvard Apparatus, Edenbridge, England) to determine $\dot{V}O_2$ and $\dot{V}CO_2$. $\dot{V}O_2$ and $\dot{V}CO_2$ was determined using the following calculations:

$\dot{V}O_2 = (\dot{V}_I \times FIO_2) - (\dot{V}_E \times FEO_2)$

$\dot{V}CO_2 = (\dot{V}_I \times FICO_2) - (\dot{V}_E \times FECO_2)$

Where $\dot{V}_I$ is the volume of inspired air and $\dot{V}_E$ is the volume of expired air. FEO_2 is the fraction of O_2 in expired air and $FECO_2$ is the fraction of CO_2 in expired air. FIO_2 is the fraction of O_2 in inspired air, and $FICO_2$ is the fraction of CO_2 in inspired air (Consolazio et al, 1963). The CO_2 and O_2 gas analysers were calibrated using atmospheric air, ultra high purity nitrogen and 15% O_2 and 5% CO_2 alpha standards from specific gases (BOC 3.0, Auckland, New Zealand).

4.3.3 Heart rate monitoring

Heart rate was measured continuously every 5 s during the main performance trials using a chest strap and a short range telemetry downloadable heart rate monitor (Polar Electro S6101, Kempele, Finland). Heart rate data was downloaded using an appropriate computer program (Polar Precision Performance software version 3.03.011; Kempele, Finland) on completion of the performance trial.

4.4 Perceptual measures

The Felt Arousal Scale (FAS; Svebak and Murgatroyd, 1985; Appendix 6) was used to measure the participants' levels of arousal/activation at a specific time during exercise. The scale ranges from 1 indicating low arousal/activation characterised by feeling bored, relaxed, calm to 6 which indicates high arousal/activation characterised by feeling angry or excited.

The Feeling Scale (FS) (Hardy and Rejeski, 1989; Appendix 5) was used to measure perceived ratings of affective valence or pleasure-displeasure before and following exercise. The FS is an 11-point scale ranging from -5 (feeling very bad), 0 (neutral), to +5 (feeling very good) with markers in between these points.

The shortened Profile of Mood States (POMS) questionnaire (Shachman, 1983) was used as a subjective assessment of mood. The POMS is an adjective check list consisting of 37 items rated on a 5 point scale that ranges from 'not at all' to 'extremely' (Appendix 4). Six factors are derived: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Fatigue-Inertia, Vigour-Activity and Confusion-Bewilderment. The questionnaire was used immediately prior to the performance time trial test, immediately post the time trial and 60 min post-exercise. An

advantage of using the shortened POMS is the ease of administration (Shachman, 1983).

The Ratings of Perceived Exertion (RPE) scale (Borg, 1973; Appendix 7) is a 15-point scale used to measure how hard the participants perceived they were cycling. The scale ranges from 6 (very, very light) to 20 (maximum) to describe the effort of the participants. The scale was administered throughout the time trial and immediately afterwards.

4.5 Dietary Control

Subjects were asked to refrain from alcohol, caffeine and tobacco in the 48-h period before the main performance trials and to refrain from exercise the day before the glycogen reduction exercise. Subjects were also asked to record their dietary intake during this 48-h period and to replicate their diet 48-h prior for all other trials (Appendix 9). Electronic scales (Wiltshire Electronic Kitchen Scale, China) were given to the participants to help record their diet accurately (diets were analysed for total energy intake and relative contributions of food types using Food Works version 5.0.1324 Xyris Software, Australia 2007). Burke et al (1996) has shown that in order to replenish glycogen stores >1 $g \cdot kg^{-1}$ body mass of carbohydrate is needed to improve exercise performance and that <1 $g \cdot kg^{-1}$ body mass carbohydrate intake would leave one in a glycogen reduced state. After the evening glycogen reduction exercise subjects were given a low carbohydrate (~1 $g \cdot kg^{-1}$ body mass) controlled meal and then underwent a subsequent overnight fast (10-12 h). This regime ensured that the participants arrived at the laboratory (on the morning of Day 2) with reduced liver and muscle glycogen stores so that the impact of carbohydrate ingestion and/or rinsing could be assessed in a fasted state; this method has been successfully used in the past (Ali et al, 2007). If there was any left-over food, the participants were asked to bring the contents back to the laboratory so that the food could be weighed the following day. If a participant had not consumed their entire meal, during diet analysis, the total energy content and macro-nutrient amounts for that day would be adjusted according to the left-over weighed amount. After the evening session, participants were advised to refrain from physical activity and consuming anything else other than water and this was to be recorded in their diaries (Appendix 10). The following morning the performance time trial ride was conducted.

4.6 Subject Control

Participants received no information of performance (performance time, heart rate and power output) other than the amount of work completed and the present amount of work relative to

the total amount to be completed. Verbal encouragement was not given to the participants during the performance time trial and music and external distracting material was removed.

4.7 Ambient temperature, humidity, and barometric pressure

Ambient temperature, humidity and barometric pressure were recorded before and after trials using an onsite weather station (Deluxe Weather Station 2111, Dick Smith Electronics, Auckland, New Zealand). The temperature was maintained within 18 to 20 ºC using the laboratory's air conditioning unit.

4.8 Experimental Environment

All experimental trials were performed in the Massey University Albany, Sport and Exercise Science laboratory. The experimental table was set up 1.5 m away from the cycle ergometer and an electric fan was placed 1 m directly in front of the cycle ergometer. The fan was set at the maximum level '4' during the trials.

4.9 Preliminary Session

During the initial preliminary session an electronically-braked cycle ergometer (model Excalibar, Lode, Groningen, Netherlands) with work load program was individually set up for the participants and then a $\dot{V}O_2$max test was conducted using appropriate software. The graded exercise test (Kuipers et al 1985) included a 5 min warm-up at 100 W. After the warm up the workload was increased by 50 W every 2.5 minutes until a heart rate of 160 beat·min^{-1} was reached. After this point, workload was increased by 25 W every 2.5 min. Gas samples (60 s) were collected at every 2.5 min stage. The test continued until participants reached volitional exhaustion. The participants were asked to signal when they could go for only one more minute and a final sample was collected. The participants' maximum power output (Wmax) achieved was recorded (Appendix 8) and they were verbally encouraged throughout the test. The Douglas bag samples were then analysed to determine the participants' $\dot{V}O_2$ peak.

After the $\dot{V}O_2$max test the participants underwent a 15 min familiarisation of the glycogen reduction exercise protocol (see section 4.10.1). The participants then performed a full familiarisation of the 1-h cycling time trial protocol (Jeukendrup et al 1997). During the performance trial the participants were introduced to the perceptual scales, oxygen uptake measures, and mouth rinse protocol. The preliminary session also familiarised the participant

to self-adjust power output (Watts). The participant was given a diet recording form and activity recording form (Appendices 9&10) for them to take away.

4.10 Main Trials

4.10.1 Glycogen reduction exercise protocol

The glycogen reduction exercise was designed to reduce the glycogen content in both type I and type II muscle fibres (Vollestad, 1992; Figure 4.2). After nude body mass was recorded the subject performed 30 min of exercise at an intensity close to 70% of their cycling maximum power output, with a pedal rate of 70 rev·min^{-1}. After the initial 30 min of exercise, the subject underwent a 2-min rest period, followed by three 50-s 'sprints' at double the resistive load, at a pedal rate of >80 rev·min^{-1}. After another 2 min rest period the participant cycled for another 45 min at 70% Wmax (at 70 rev·min^{-1}) to further reduce glycogen in type I fibres.

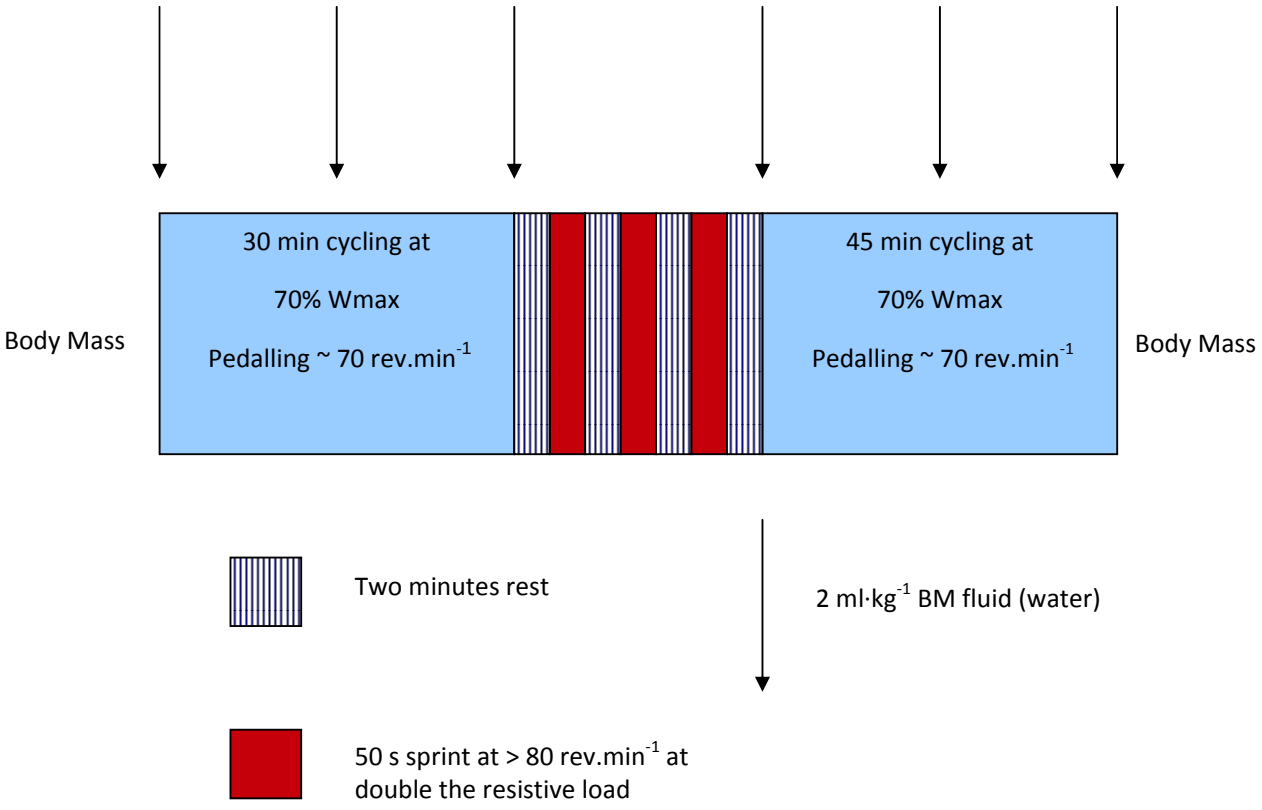

Figure 4.2: Diagrammatic representation of the glycogen reduction exercise protocol

The subjects were provided with 2 ml·kg^{-1} body mass of water before and after the session and every 15 min of exercise to offset severe dehydration. Nude body mass was recorded after the exercise and the participant was given a pre-packaged low carbohydrate meal to take home and consume (~1 g·kg^{-1} body mass) (Table 4.1).

Table 4.1 Typical meal for a subject with a body mass of 70 kg

Foodstuff (Based on 56.9kJ.kg^{-1} at 70kg standardised = 3981.9k)	**Weight of food (g)**	**Energy content (kJ)**	**CHO content (g)**
Chicken breast (uncooked)	250	1150	0
Korma sauce (uncooked)	210	1066.8	16.8
Rice (cooked)	188	1022.72	52.3
Protein shake	25	370	1.0
Vegetable oil	10 (2 tsp)	372.4	0
Total	**673**	**3981.9**	**70.1**

4.10.2 Performance time trial protocol

Participants arrived on the same day and at the same time in the morning each week for their performance trials. Following the initial blood sample, resting heart rate was taken. After a brief warm-up (5 min at 40% Wmax), the timer was started and subjects performed a pre-determined amount of work as fast as possible using the cycle ergometer, where the total work was based on the following formula:

Total work (J) = 0.75 · Wmax · 3600 s

The total amount of work (J) to be performed was calculated by assuming that the participants could cycle at 75% of their maximum power output (Wmax) for 60 min (Jeukendrup et al 1996). Participants were asked to pedal at 90 rev·min^{-1}. Power output during the performance trial was self-selected. The changes of power output were recorded by the investigator and the total amount of work to be performed was recalculated based on the power output at that point in time. Key performance indicators were performance time and mean power output over the whole test.

Perceptual scales (RPE, FAS and FS) were shown to the participant, and subsequent responses recorded, following every 25% of exercise completed. The shortened Profile of Mood States (POMS) questionnaire was administered pre and post-exercise. During the time trial rides heart rate was recorded every 5 s of exercise and oxygen uptake was measured every 20% of exercise completed (Figure 2.3). The trial solution was administered every 12.5% of exercise completed. Using indirect calorimetry calculations, energy expenditure and fat and carbohydrate utilisation were estimated during exercise.

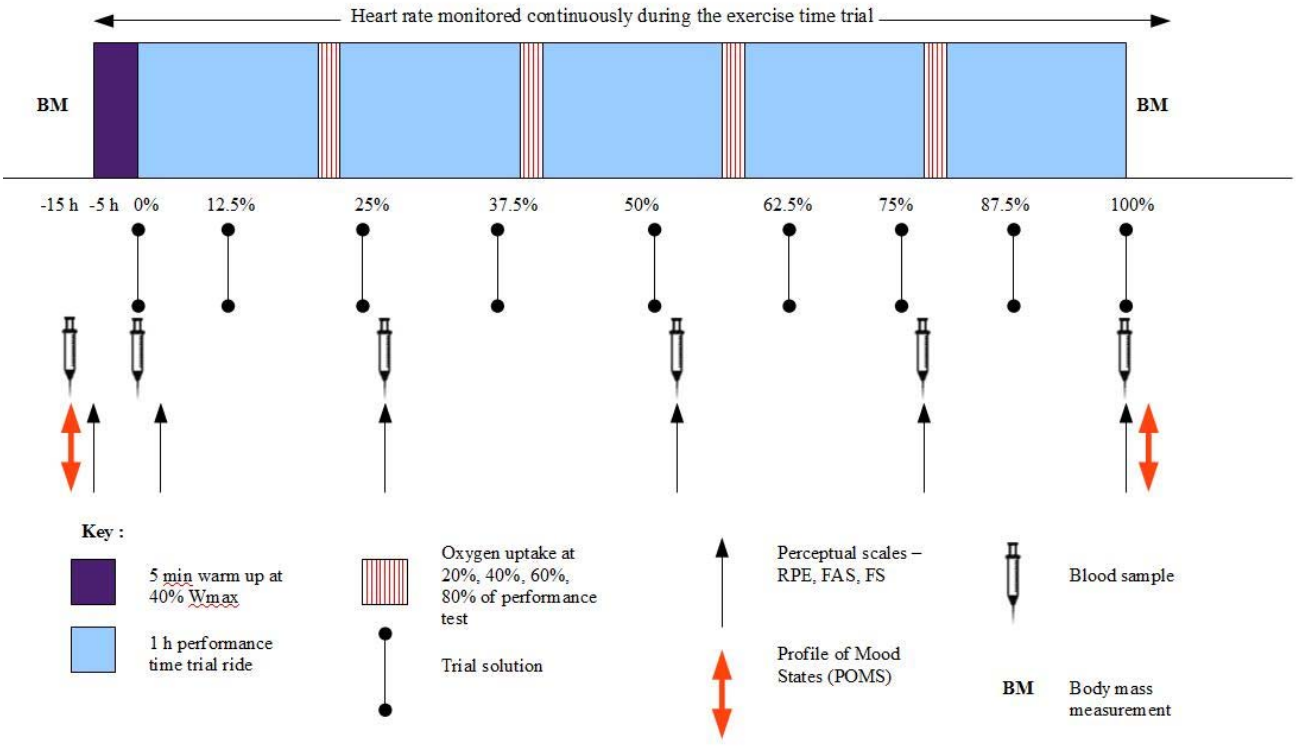

Figure 4.3 Diagrammatic representation of the cycling performance trial

Subjects underwent four main trials separated by at least seven days and the experimental conditions were as follows:

A - Carbohydrate mouth rinse (CHOR)
B - Placebo mouth rinse (PLAR)
C - Carbohydrate ingestion (CHOI)
D - Placebo ingestion (PLAI)

In Trial A, a 15% carbohydrate solution was used for rinsing and in Trial C a 7.5% carbohydrate solution was used for ingestion. The 15% carbohydrate mouth rinse solution has been used previously (Gant et al 2010). The 7.5% carbohydrate solution has also been used previously (Ali et al 2011). The placebo solutions (Trials B and D) were taste and colour matched and contained 0% carbohydrate and artificial sweeteners. The solutions had a mandarin flavour and were made at the Massey University Albany Food Technology laboratory (Table 4.2) and were stored in the laboratory food refrigerator (Fisher &Pykell, c450, New Zealand). Trial solution administration and recipes were made up according to methods used previously (Ali et al 2011).

Table 4.2 Recipes of the trial solutions

Sample Code	Sucrose (g/L)	Maltodextrin (g/L)	Aspartame (g/L)	Citric Acid (g/L)	Sodium (g/L)	Chloride (mmol/L)
PLA	0	0	0.41	0.19	0	0
CHO Rinse	118	32	0	0.19	1.07	18.26
CHO Ingestion	59	16	0	0.19	1.07	18.26

During the ingestion trials 1.5 ml·kg^{-1} body mass solutions were consumed using a sipper bottle which has been used similarly in previous studies (Gant et al 2010; Pottier et al 2008). The participant was informed to finish the solution when it was given to them. The trial solution was given every 12.5% of exercise completed. During the mouth rinsing trials, the participants were required to rinse 0.33 ml·kg^{-1} body mass solutions and these were given to the participant in a plastic volumetric syringe (Omnifix 50/60ml Luer; Germany), (Pottier et al 2008). Participants self-administered the mouth rinse and were asked to swirl the solution in their mouth for 8 s (Figure 4.4; Pottier et al, 2008; Chambers et al, 2009). After rinsing, participants expectorated all of the solution into a pre-weighed container which was then accurately measured using electronic scales accurate to 0.0001 g (Sartorius LE3235, Germany) (Rollo et al, 2010). The mouth rinse was also administered every 12.5% of exercise completed (Figure 4.4).

Figure 4.4 Mouth rinse application

4.11 Blood Sampling and Dispensing

Blood samples were taken on the day of the performance trial via an indwelling cannula (Venflon, 1618G, Ohmedia, Hatfield, Herts). The cannula was inserted into an antecubital vein and was kept patent by frequent flushing with sterile saline (Figure 4.5). An initial pre-performance trial blood sample was taken and samples were then collected every 25% of exercise completed.

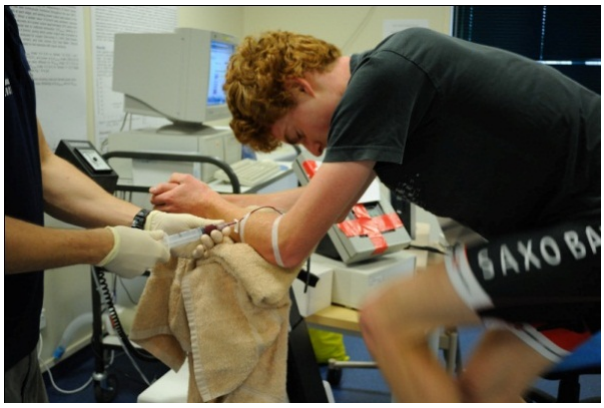

Figure 4.5 Blood sampling during performance time trial.

Twelve millilitres of blood was collected at each sample point. Six millilitres of the sample was collected in an ethylenediaminetetraacetic acid (EDTA) tube and 4 ml was collected in a heparinised tube. To determine the hematocrit (packed cell volume), three microhematocrit tubes were filled with heparinised blood and were then centrifuged at 10,000 rev·min^{-1} for 5 min (Haematocrit 210, Hettich, Germany). Linearity was measured by a microhematocrit reader (Hawkesley, Cambridge, England) and hemoglobin was measured using an automated method (Hemocue, AB, Angelholm, Sweden).

The vacutainer tubes were centrifuged at 1500 G (Hanil, MF50, Korea) for 10 min at 4°C. Each sample was sub-aliquoted into 5 labelled tubes, each containing at least 150 µl whenever possible in labelled (participant code, date, sample time, Heparin/EDTA) tubes with screw caps. The sub-aliquots were then placed into a storage box in an ice bath. When a storage box was full, it was placed on dry ice. When all of the aliquots were complete for a participant, the storage boxes were placed in a -80°C freezer (Thermaforma 929, Ohio, USA) for later analysis of metabolites and hormones.

4.12 Blood Analysis

Lactate was analysed via an enzymatic method using lactate oxidase (LOD) (Roche Diagnostics GmbH. Switzerland; Flexor E, Vitalab Netherlands). Plasma glucose was determined using a hexokinase method (Roche Diagnostics, Basel, Switzerland; Flexor E, Vital Scientific NV, 6956 AV Spankeren/Dieren, The Netherlands). Free fatty acids (FFA) were assayed by an ACS-ACOD enzymatic method and NEFA concentrations were then obtained by measuring absorbance of the blue colour (Wako pure chemical Industries, Ltd. Osaka, Japan and Flexor E, Vitalab scientific NV, 6956 AV Spankeren/Dieren, The Netherlands).

Insulin and c-peptide were analysed using Mercodia ultrasensitive insulin and C-peptide ELISA (a solid phase two-site enzyme immunoassay) kits, respectively. This method is based on the direct sandwich technique in which two monoclonal antibodies are directed against separateantigenic determinants on the insulin molecule (Mercodia AB, Sylveniusgatan 8A,SE-754 50 Uppsala, Sweden).The samples were performed in duplicate and the optical density was read at 450 nm.

4.13 Statistical Analysis

Data collected for most of the variables were compared using a two-way analysis of variance (ANOVA) with repeated measures (SPSS version 16.0. Chicago, IL) to examine main effects of i) treatment (PLAR, PLAI, CHOR, CHOI) and ii) time (pre time trial and every 25% of exercise completed) and iii) interaction of treatment x time. Mauchly's test for sphericity was applied to the data to examine if sphericity was violated. When sphericity was violated the

Huynh-Feldt estimate was used to correct the data. One-way ANOVA was used to examine performance time to completion and overall POMS scores and plasma volume. When significant differences between the interventions were identified by ANOVA, post-hoc Student's t-test, using the Holm-Bonferroni adjustment, were performed. Correlations between variables were examined using simple linear regression equations and reported as Pearson's correlation coefficient (r). A small (weak) correlation was defined as ± .10 to ± .29, medium (moderate) correlation as ± .30 to ± .49 and large (strong) as ± .50 to ± 1.00 and the level of significance was accepted at $P<0.05$(Cohen, 1988). Data is presented as means ± SD (unless otherwise indicated). Practical significance was reported using effect sizes calculated from Cohen's d. A large effect size was determined as 0.8, medium as 0.5 and small as 0.2 (Vincent et al 1999; Cohen et al 1988).

5.0 RESULTS

5.1 Subject characteristics

The physiological and training characteristics of the participants are shown in Tables 5.1 and 5.2, respectively. There was a range of $\dot{V}O_2$peak scores among the participants (45.0 – 61.8 ml·kg·min^{-1}; Table 5.1) and training levels ranged from recreational cyclists to triathletes (Table 5.2).

Table 5.1 Physiological characteristics of subjects.

Subject	Age (Years)	Weight (kg)	Height (m)	$\dot{V}O_2$peak (ml·kg^{-1}·min^{-1})
A	27	67.65	1.74	54.1
B	19	61.80	1.78	61.8
C	29	78.45	1.76	52.1
D	54	77.65	1.86	45.5
E	26	73.50	1.75	50.0
F	20	65.50	1.81	45.0
G	27	77.40	1.85	60.0
H	40	68.15	1.82	58.0
Mean	**30.25**	**71.3**	**1.79**	**53.3**
SD	**11.5**	**6.3**	**5.0**	**6.3**

Table 5.2 Training activity of the subjects.

Subject	Training Type and time spent training per week
A	Road cycling 10 – 12 h
B	3 x 90 min soccer, 20 min indoor soccer, running 3 h week, cycling 2 h
C	Road cycling 6 h, Mountain biking 3 – 6 h
D	Road cycling 9 h, occasional mountain bike ride 3 h
E	Swimming 4 h, road cycling 4 – 5 h, running 2 h
F	Road cycling 15 – 20 h, mountain biking 3 h occasionally
G	Road cycling and mountain biking 10 – 15 h
H	Road cycling 10 – 14 h

5.2 Performance parameters

5.2.1 Mean Power Output

Mean power output (Watts) was recorded every 12.5% of the test completed. The four trials include: PLAR = Placebo rinse, PLAI = Placebo ingestion, CHOR = Carbohydrate rinse, CHOI = Carbohydrate ingestion). There was a main effect of treatment for power output ($F_{3,}$

$_{21}$= 5.08; P=0.002). There was also a main effect of time, which significantly changed from the start of exercise (0%) to the end of exercise (100%) for mean power output ($F_{8, 56}$= 28.36; P=0.000). Post hoc analysis revealed that CHOI had a significantly higher mean power output relative to the other trials (P<0.05; Figure 5.1).

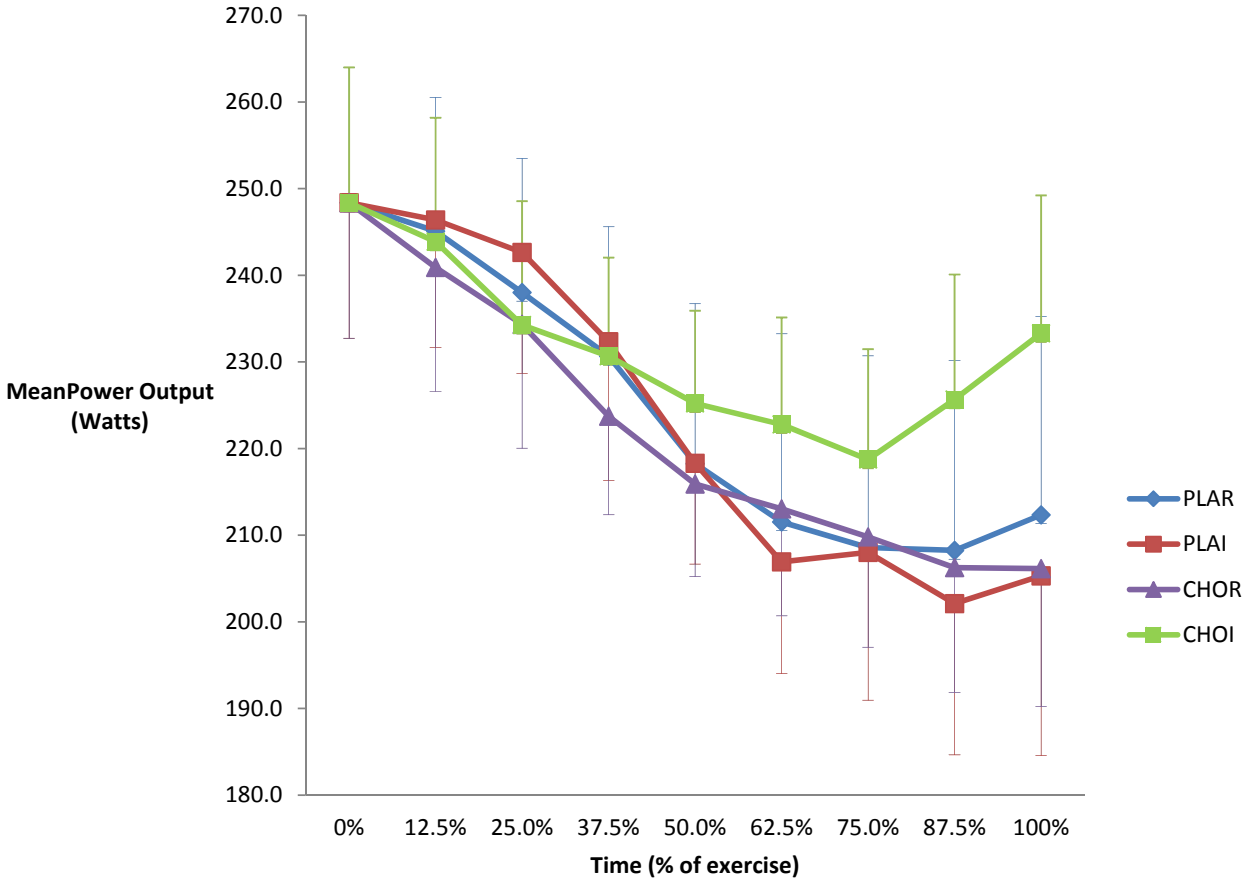

Figure 5.1 Mean Power Output during the performance time trial (mean ±SD; n= 8).

5.2.2 Mean Performance Time

Figure 4.2 shows the mean performance time for all trials. The data shows a 4.2-4.6% non-significant difference in performance time in the CHOI trial (3919 ±307.4 s; Figure 5.2) relative to the other trials (4015 – 4108 s; $F_{3, 28}$= 0.703 P= 0.55). Although this difference was not statistically significant, from a practical significance perspective, Cohen's d values of

between 0.26 and 0.67 indicate small to medium effect sizes between CHOI and the other trials.

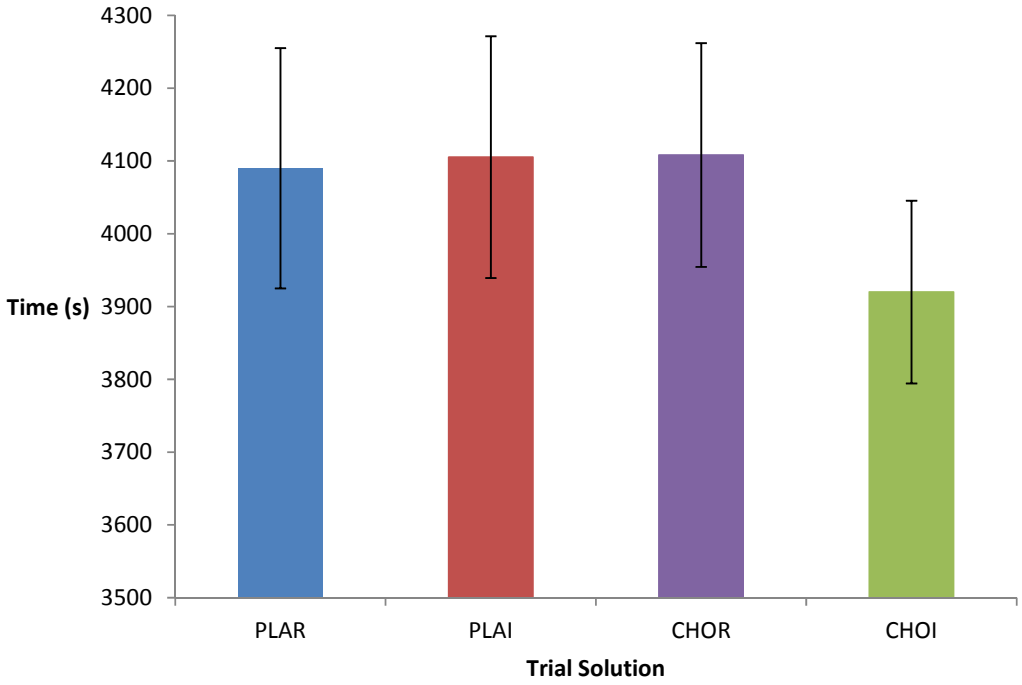

Figure 5.2 Mean performance time (s) of the time trials (mean ±SD; n= 8).

5.3 Expired air parameters

Table 5.3 provides a summary of the expired air parameters. There were no main effects of treatment ($F_{3, 21}$= 2.24; P= 0.67) or time ($F_{3, 21}$= 4.28; P=0.06) for $\dot{V}O_2$ (ml·kg^{-1}·min^{-1}) during the performance trials (Table 5.5). However, $\dot{V}O_2$ appeared to be ~6% lower in CHOI trial (39.6 ± 2.4 ml·min^{-1}) relative to other trials (42.2-43.6 ml·min^{-1}; Cohen's d = 0.5 – 0.8). Nevertheless, the relative exercise intensity (%$\dot{V}O_2$peak) was maintained at around 75-80% in all trials with no main effect of treatment ($F_{3, 21}$= 8.01; P= 0.73) or time ($F_{3, 21}$= 5.70; P=0.16).

Table 5.3 Mean oxygen uptake and relative exercise intensity. Mean (±SD) values of the first 20% to 80% of exercise during the trials are shown (n=8).

Parameter	Trial	Time (% of exercise)				Mean of trial
		20	40	60	80	
$\dot{V}O_2$	PLAR	45.3 ± 8.8	46.0 ± 10.7	41.0 ± 6.9	41.9 ±6.4	**43.6 ± 2.4**
($ml \cdot kg^{-1} \cdot min^{-1}$)	PLAI	43.5 ± 7.5	44.3 ± 10.0	39.2 ± 9.2	42.6 ± 9.1	**42.4 ± 2.2**
	CHOR	41.1 ± 8.8	42.0 ± 7.2	42.9 ± 9.8	42.6 ± 6.9	**42.2 ± 0.8**
	CHOI	40.8 ± 4.4	41.3 ± 4.0	40.0 ± 5.3	36.1 ± 5.8	**39.6 ± 2.4**
	Mean	**42.7**	**43.4**	**40.8**	**40.8**	
% $\dot{V}O_{2\ peak}$	PLAR	83.3 ± 10.8	82.1 ± 8.9	77.2 ± 11.2	79.0 ± 10.9	**80.4 ± 2.8**
	PLAI	81.6 ± 10.0	82.7 ± 13.2	73.5 ± 14.0	79.7 ± 13.1	**79.4 ± 4.1**
	CHOR	76.9 ± 6.7	78.5 ± 11.9	75.2 ± 7.5	68.2 ± 11.2	**74.4 ± 4.5**
	CHOI	77.4 ± 16.6	78.8 ± 10.8	80.4 ± 15.8	80.1 ± 10.6	**79.2 ± 1.4**
	Mean	**79.8**	**80.5**	**76.6**	**76.8**	

There were no main effects for the respiratory exchange ratio (RER) between trials for treatment ($F_{3, 21}$= 2.40; P=0.39) however, there was a time effect during the performance trials (Figure 5.3; $F_{3, 21}$= 2.85; P= 0.03).

Energy expenditure rates (EE_R) were estimated through indirect calorimetry equations. There were no main effects of treatment ($F_{3, 21}$= 2.21; P=0.82) or time ($F_{3, 21}$= 4.80; P=0.74) for estimated energy expenditure.

There were no main effects of treatment ($F_{3, 21}$= 1.60; P=0.09) or time ($F_{3, 21}$= 1.40; P=0.40) for carbohydrate oxidation (CHO EE_R). However, CHO EE_R appeared to be ~15% higher in CHOI trial (30.3 ± 3.9 $kCal \cdot min^{-1}$) relative to other trials (19.9 -25.7 $kCal \cdot min^{-1}$; Cohen's d = 0.45 – 0.78). The data also shows that CHOI was ~5% lower in fat oxidation values (Fat EE_R; 28.0 ± 4.4 $kCal \cdot min^{-1}$) compared to the other trials (29.4-39.8 $kCal \cdot min^{-1}$; $F_{3, 21}$= 1.9; P=0.29). Although this difference was not statistically significant, from a practical

significance perspective, Cohen's d values were between 0.5 and 0.92 indicating medium to large effect sizes between CHOI and the other trials.

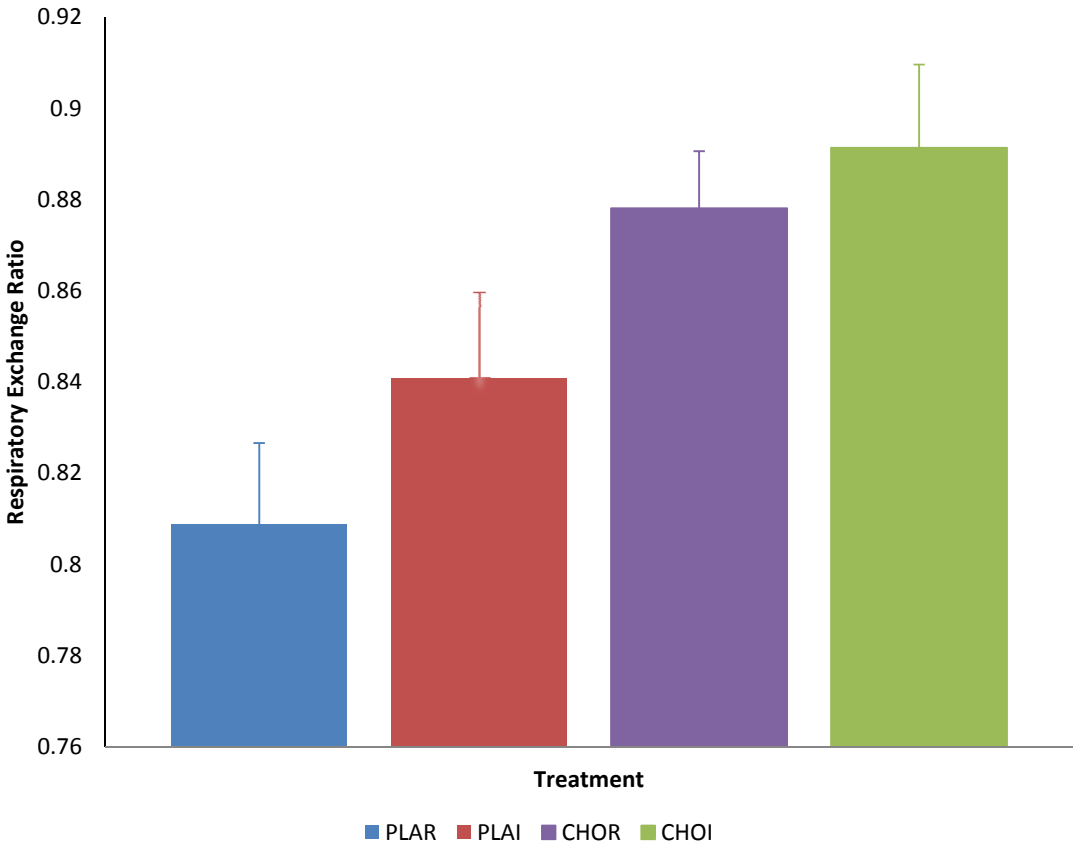

Figure 5.3 Estimated Respiratory Exchange Ratio during exercise. (Mean ±SD; n=8; P=0.39)

5.4 Blood Analyses

5.4.1 Plasma glucose

There was a main effect of treatment for plasma glucose concentration, ($F_{3, 21}$= 11.76, P<0.001; Figure 5.4) with highest values in CHOI relative to the other trials. There was also a significant interaction of treatment and time ($F_{12, 84}$ = 3.73, P<0.01). Subsequent analysis

showed that the CHOI trial had significantly higher levels at 75% of exercise (5.42 ±0.84 mmol•L^{-1}) compared to the other treatments (4.20-4.76 mmol•L^{-1}). CHOI also had significantly higher levels at 100% of exercise (5.94 ±1.24 mmol•L^{-1}) compared to the other treatments (3.92-4.67 mmol•L^{-1}; P<0.05; Cohen's d = 0.3 – 0.6).

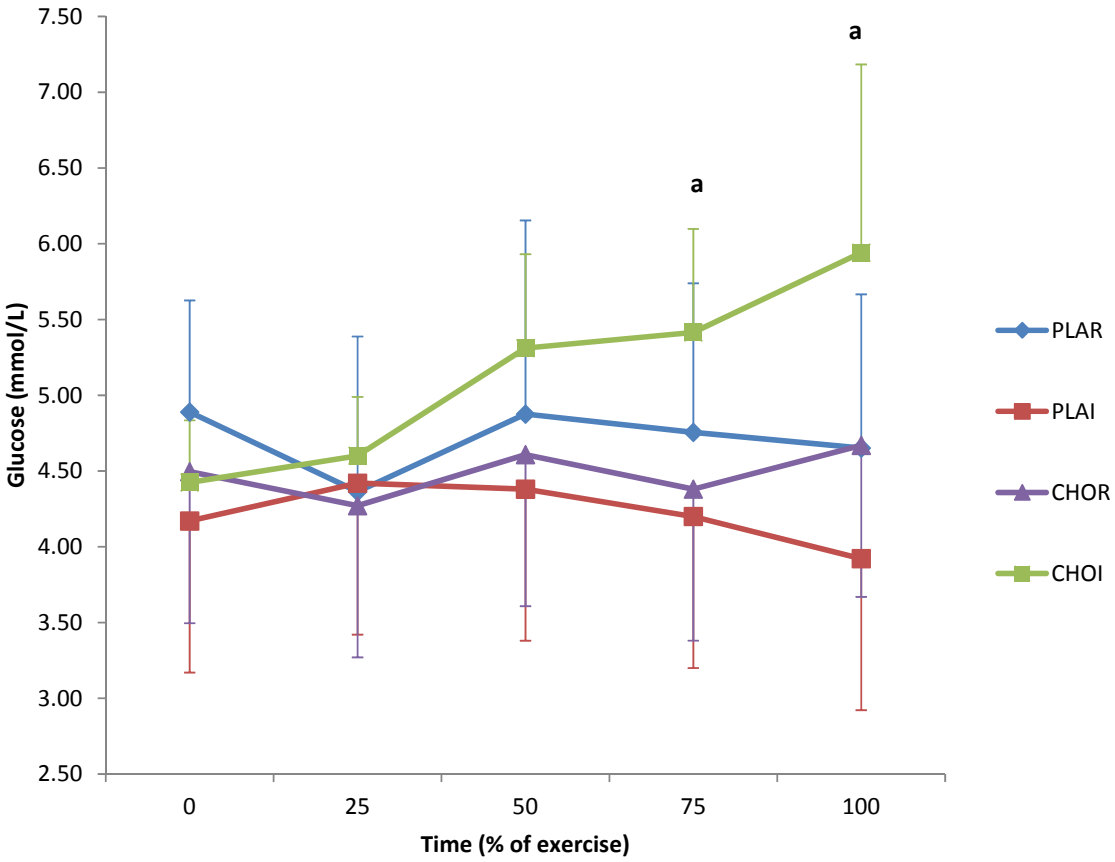

Figure 5.4 Plasma glucose concentrations (mmol·L^{-1}) during the cycling time trial for carbohydrate mouth rinse (CHOR), carbohydrate ingestion (CHOI), placebo mouth rinse (PLAR) and placebo ingestion (PLAI) trials (n=8 Mean ± SD). (**a** = CHOI significantly higher than other time trials at the time point, P<0.05).

5.4.2 Insulin

There was a main effect of treatment for insulin concentrations ($F_{1, 7} = 3.7$; P=0.001; Figure 5.5) with highest values in CHOI relative to the other trials. There was also a significant time effect where insulin decreased steadily during exercise ($F_{4, 28} = 4.7$; P<0.001). Post-hoc analyses revealed that insulin was significantly higher in CHOI at 50% and 75% of exercise compared to the other treatments (P<0.05; Cohen's d = 0.12 – 0.22).

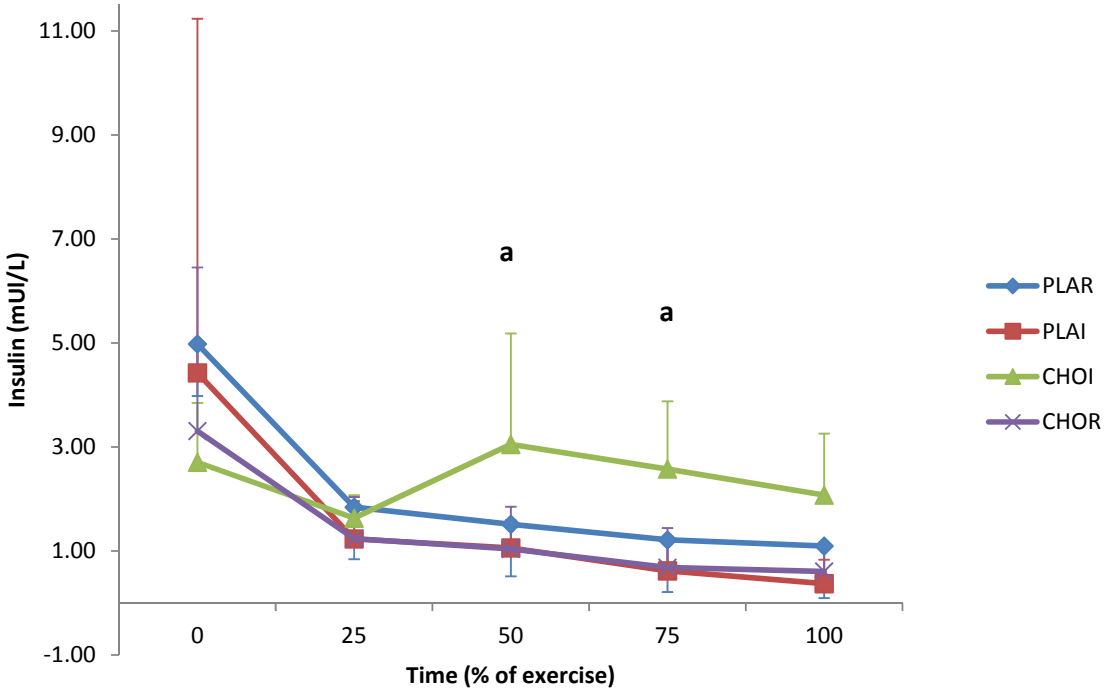

Figure 5.5 Plasma insulin concentrations ($mUl \cdot L^{-1}$) during the cycling time trial for carbohydrate mouth rinse (CHOR), carbohydrate ingestion (CHOI), placebo mouth rinse (PLAR) and placebo ingestion (PLAI) trials (n=8 Mean ± SD). (**a** = CHOI significantly higher than other trials at the time point, P<0.05).

5.4.3 C-peptide

There was a main effect of treatment for C-peptide ($F_{2, 7.5} = 4.517$; P<0.03) with highest concentrations in CHOI relative to the other trials (P<0.05). Mean concentrations were

maintained at near resting values in the CHOI trial but decreased markedly in the other three trials (PLAR; 2.86 ±1.38 mmol•L^{-1}, PLAI; 2.24 ±1.32 mmol•L^{-1}, CHOR; 2.32 ±1.12 mmol•L^{-1} or CHOI; 2.81 ±0.84 mmol•L^{-1}; Figure 5.6; Cohen's d = 0.2). There was also a significant main effect of time ($F_{3, 21}$= 2.70; P<0.001) with values decreasing from pre- to post-exercise.

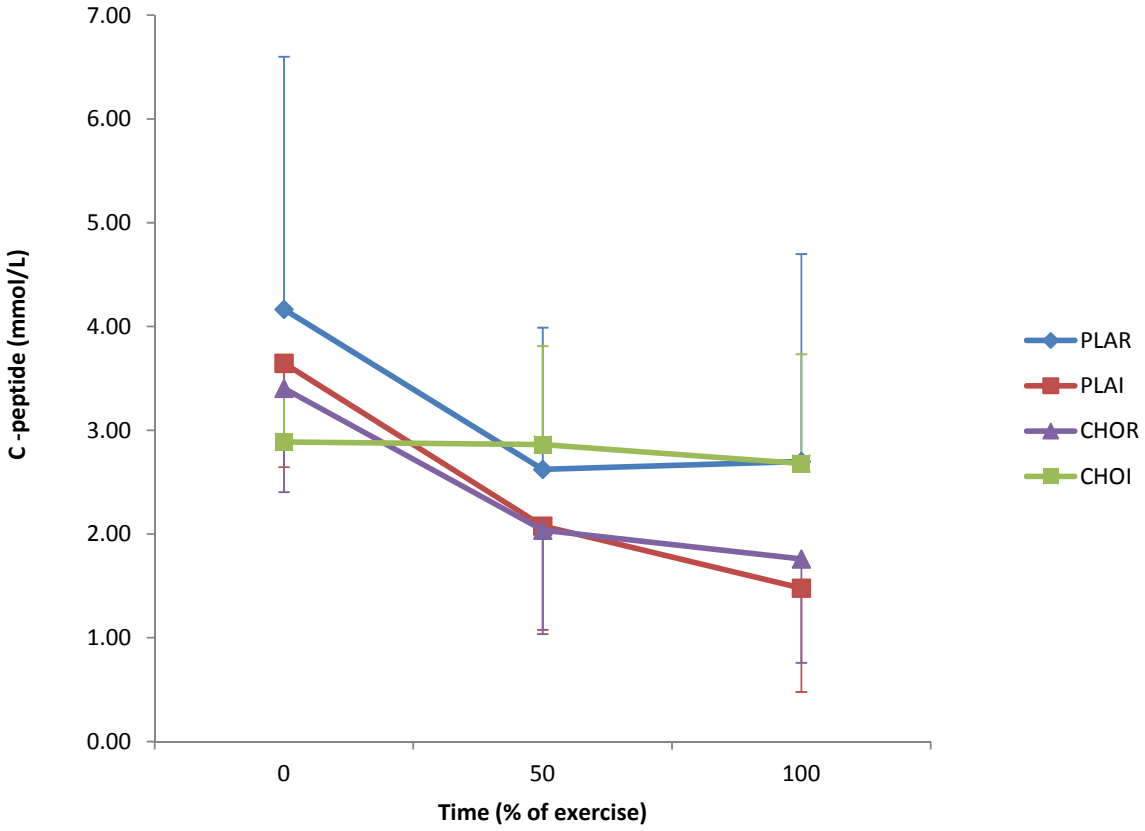

Figure 5.6 C-peptide concentrations (mmol·L^{-1}) during the cycling time trial for carbohydrate mouth rinse (CHOR), carbohydrate ingestion (CHOI), placebo mouth rinse (PLAR) and placebo ingestion (PLAI) trials (n=8 Mean (± SD).

5.4.4 Circulating Lactate

There were no significant main treatment ($F_{3, 21}$= 3.50; P=0.72) or time effects ($F_{3, 21}$= 8.50; P= 0.38) found in circulating lactate concentrations. There was however an interaction of treatment x time ($F_{6, 42}$= 1.76; P= 0.002). Statistical differences were found at 100% of the time trial with CHOI significantly higher (4.45 ± 2.23 mmol·L^{-1}) than PLAR (3.68 ± 1.69 mmol·L^{-1}; P<0.05 vs. PLAI 3.15 ± 1.70 mmol·L^{-1}; P=0.03 vs. CHOR 2.55 ± 1.19 mmol·L^{-1}; P<0.05; Figure 5.7).

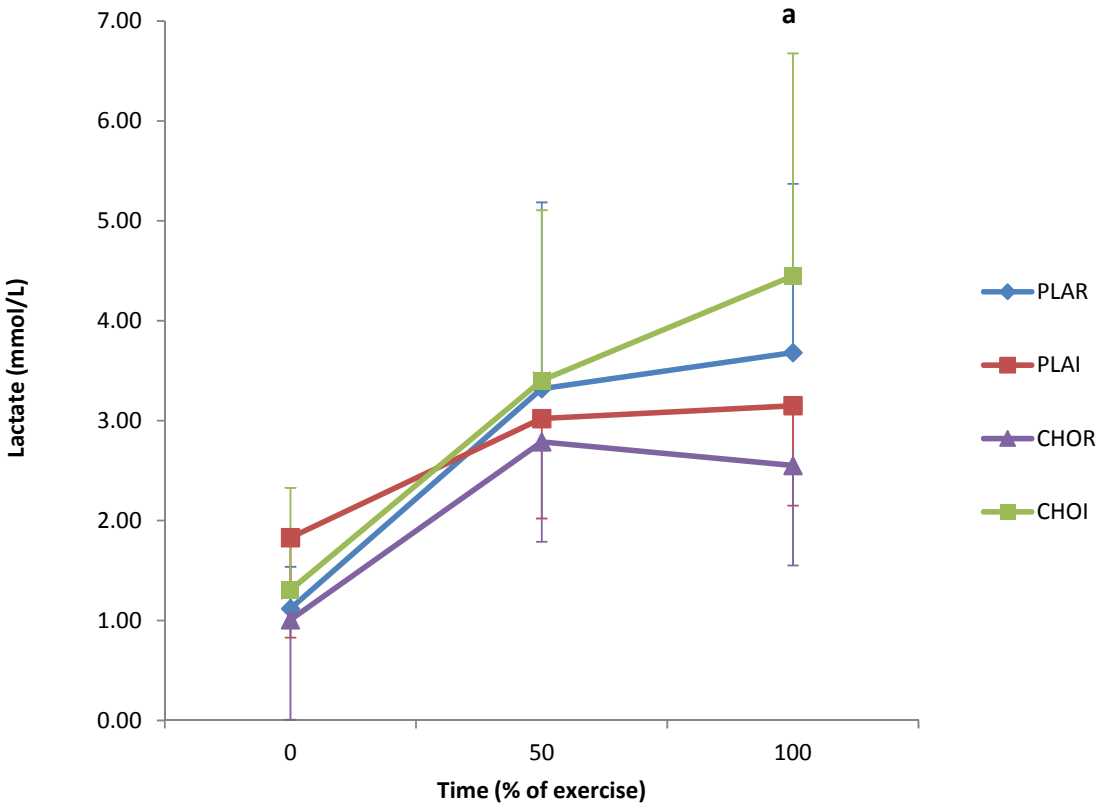

Figure 5.7 Circulating Lactate concentrations (mmol·L^{-1}) during the cycling time trial for carbohydrate mouth rinse (CHOR), carbohydrate ingestion (CHOI), placebo mouth rinse (PLAR) and placebo ingestion (PLAI) trials (n=8). (**a** = CHOI significantly higher than other trials at the time point, $P<0.05$; Mean ± SD).

5.4.5 Free fatty acid

There were no significant differences found in free fatty acid (FFA) levels for treatment ($F_{3, 21}= 1.26$; $P<0.05$). Mean concentrations increased markedly during exercise in PLAR, PLAI and CHOR compared to CHOI however, there was no statistical significance (CHOI 0.36 ±0.15 mmol·L^{-1} compared to PLAR 0.38 ±0.24 mmol·L^{-1}; PLAI 0.44 ±0.19 mmol·L^{-1}; CHOR 0.43 ±0.43 mmol·L^{-1}; Figure 5.8; Cohen's d = 0.13-0.43).

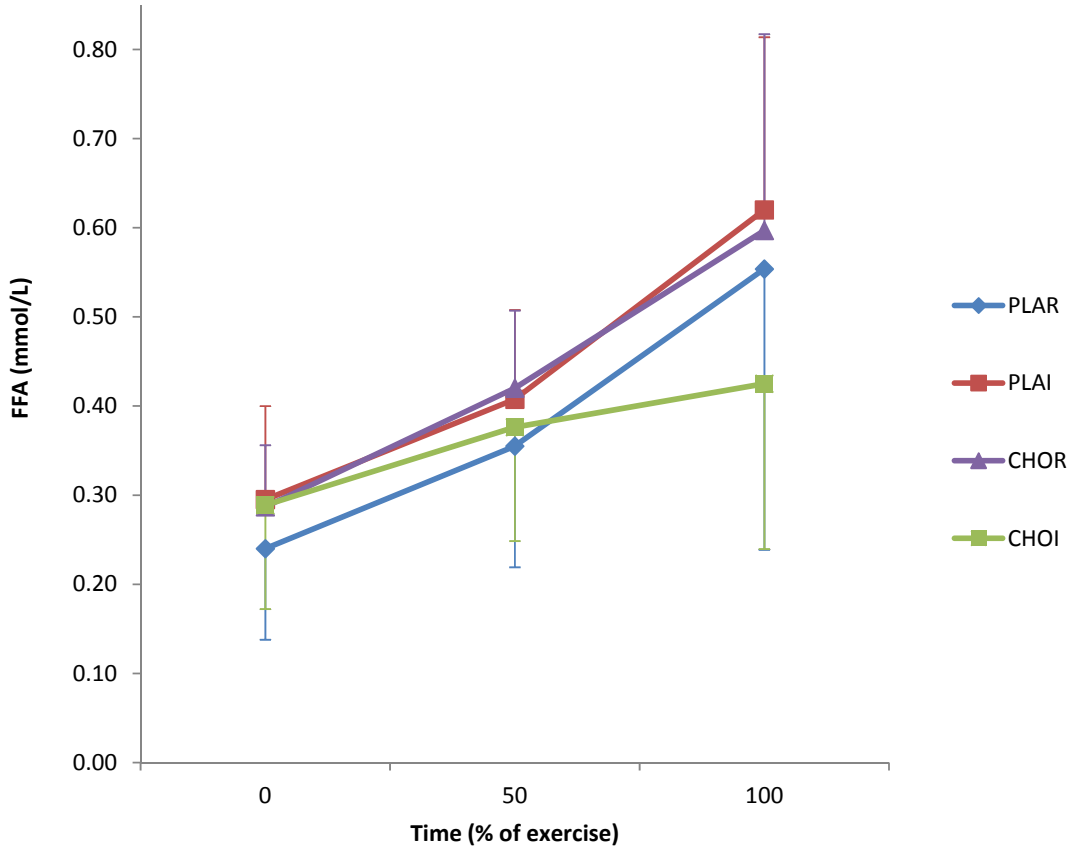

Figure 5.8 Free fatty acid (FFA) concentrations ($mmol \cdot L^{-1}$) during the cycling time trial for carbohydrate mouth rinse (CHOR), carbohydrate ingestion (CHOI), placebo mouth rinse (PLAR) and placebo ingestion (PLAI) trials (n=8 Mean ± SD).

5.5 Perceptual data

There were no significant main effects or interaction effects ($F_{3, 21}$= 7.50; P =0.39) for the overall profile of mood states (POMS) or any of these subcomponent interaction scores of fatigue ($F_{3, 21}$= 7.65; P=0.372; Figure 5.9), and vigour ($F_{3, 21}$= 6.96; P=0.56; Figure 5.10) compared to the other three trials from pre-time trial to immediately post the time trial.

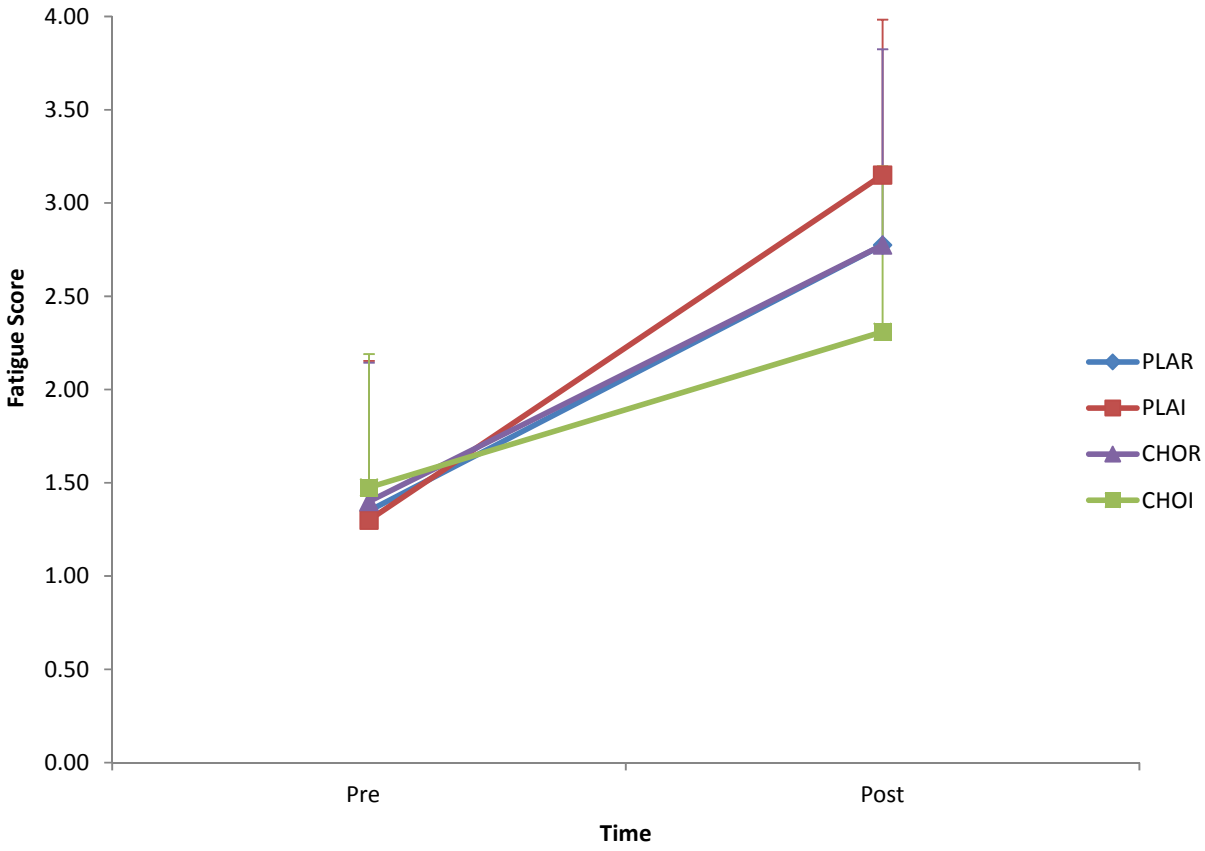

Figure 5.9 Overall profile of mood states (POMS) fatigue subscale results before and immediately post the cycling time trial. Mean (± SD) n=8.

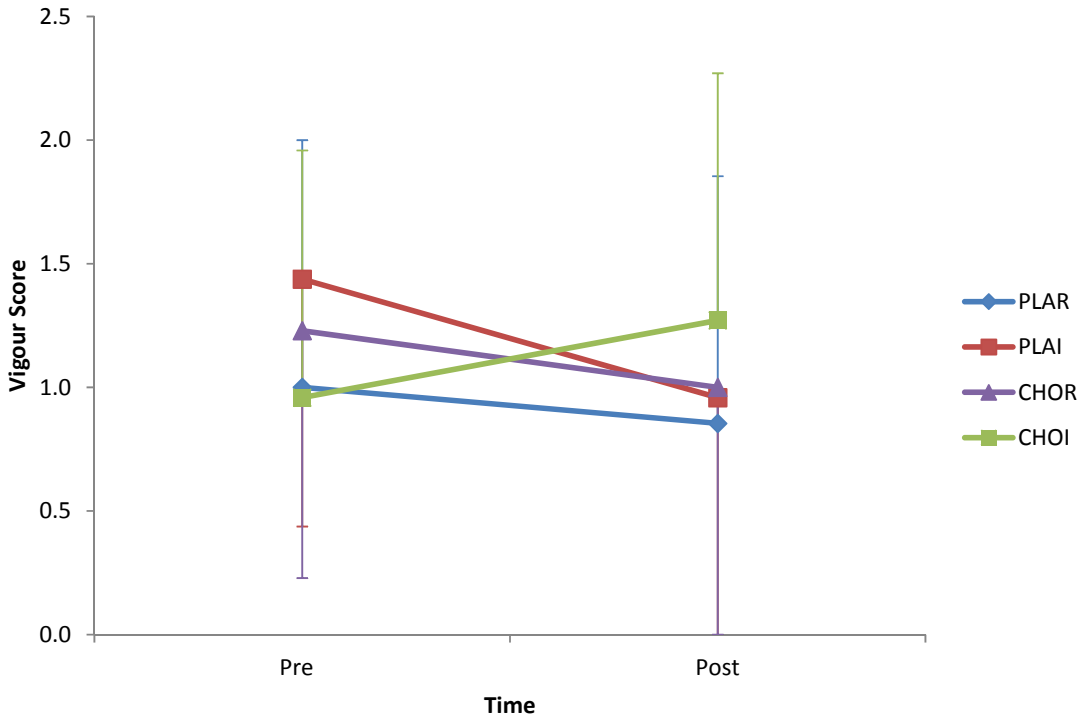

Figure 5.10 Overall profile of mood states (POMS) vigour subscale results before and immediately post the cycling time trial. Mean (± SD) n=8.

Further analysis of the POMS data showed that post exercise fatigue sub scores and post exercise vigour sub scores were strongly and inversely correlated ($r = -0.567$; $P<0.01$). There was also a weak non-significant, inverse correlation between the overall fatigue sub score and blood glucose ($mmol \cdot L^{-1}$), **(**$r = -0.221$; $P=0.08$).

Table 5.4 Subjective perceptions of perceived activation (Felt Arousal Scale) and pleasure/displeasure (Feeling Scale) experienced by participants pre exercise and during exercise for each trial mean (±SD) n = 8.

		Time (% of exercise)						
		Pre	**0**	**25**	**50**	**75**	**100**	**Mean**
Felt Arousal Scale	**PLA R**	2.4±1.1	2.9±1.0	3.0±0.8	3.6±1.3	3.5±1.3	4.0±1.8	**3.2±0.6**
	PLA I	2.1 ±1.5	2.7±1.7	3.0±1.2	3.4±1.7	3.7±1.6	4.4±1.7	**3.2±0.8**
	CHO R	2.3 ±1.1	2.6±0.5	3.4±0.8	3.1±0.9	3.6±1.3	4.4±1.0	**3.2±0.8**
	CHO I	2.1 ±1.1	3.1±1.3	3.0±0.8	2.9±0.7	3.9±0.9	4.6±1.0	**3.3±0.8**
	Mean	**1.7±0.6**	**2.0±1.0**	**2.0±1.2**	**2.2±1.2**	**2.5±1.3**	**2.9±1.6**	
		Pre	**0**	**25**	**50**	**75**	**100**	**Mean**
Feeling Scale	**PLA R**	1.3 ±1.3	0.0±1.2	-0.5±1.3	-0.8±1.5	-1.5±1.6	-0.6±2.3	**-0.4±0.9**
	PLA I	0.5 ±1.5	0.3±1.2	-0.5±1.1	-0.5±0.8	-1.0±0.8	-1.4±1.8	**-0.4±0.7**
	CHO R	1.0 ±1.3	0.0±1.3	-0.1±1.4	-0.6±1.3	-0.9±1.1	0.3±2.4	**-0.1±0.7**
	CHO I	0.6 ±0.7	0.9±0.6	-0.4±1.5	-0.6±0.8	-0.6±1.7	0.0±2.6	**0.0±0.6**
	Mean	**0.8±1.1**	**0.3±1.0**	**-0.4±1.2**	**-0.6±1.2**	**-1.0±1.5**	**-0.4±2.1**	

Figure 5.11 shows the mean RPE data over the four trials. There were no significant differences observed between trials for ratings of perceived exertion. There was a significant time effect from the start of exercise (0%) to the end of exercise (100%) where RPE increased over time ($F_{4, 20}$= 7.3; P=0.01). There were no time or treatment effects for affective valence (FS) and felt activation (FAS; Table 5.4).

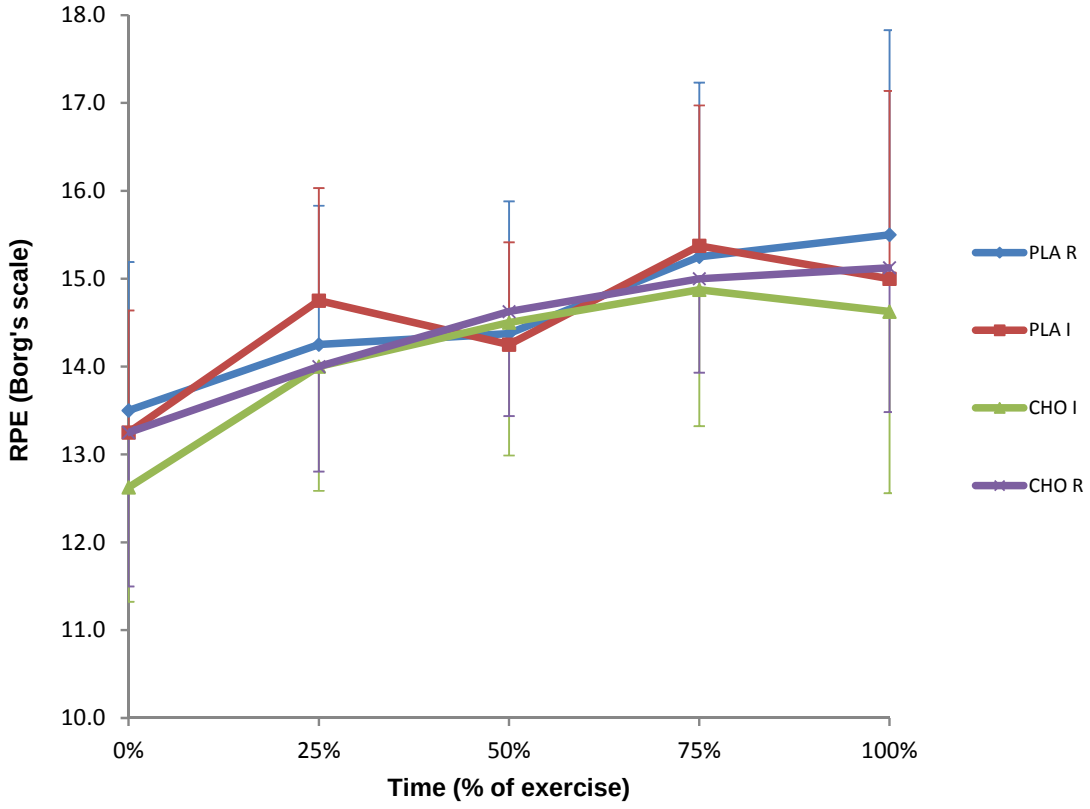

Figure 5.11 Ratings of perceived exertion (RPE) during trials. Mean (±SD) scores taken every 25% of exercise are shown (n = 8).

5.6 Indicators of physiological data (Plasma volume, Body mass, Heart rate)

A summary of the indicators of physiological demands are presented in Table 5.5 and Figures 5.12 and 5.13.

Table 5.5 The change in plasma volume relative to exercise (mean ±SD; PV data, n=8). (Significant differences: **a**= from 25% to 100% of exercise; P<0.05; **b** = from Pre-exercise; **c** = from ingestion trials; P<0.01)

		Time (% of exercise)					
Plasma Volume Decrease (%)		**0**	**25**	**50**	**75**	**100**	**Mean**
(Relative to pre-Exercise)	**PLA R**	13.0±4.8	6.4±3.9	10.6±3.0	11.3±3.1	10.4±3.6	**14.7±2.4**
	PLA I	7.1±5.5	7.0±3.9	9.5±4.4	9.2±4.4	10.3±5.0	**10.9±1.5**
	CHO I	10.4±3.6	7.3±2.1	8.1±1.6	7.5±1.9	10.3±2.8	**11.5±1.5**
	CHOR	9.0±6.1	9.2±4.4	12.6±4.2	14.3±4.5	12.7±4.6	**12.7±2.3**
	Mean	**9.9±2.5**	**7.5±1.2**	**10.2±1.9**	**10.6±2.9**	**10.9±1.2**	

There were no significant differences observed between trials for decrease in plasma volume as a percentage of total blood volume (Table 5.5). Body mass significantly decreased following the two mouth rinse trials (PLAR 1.31 ±0.46 kg and CHOR 1.23 ±0.28 kg) than the ingestion trials (PLAI 0.42 ±0.21 kg and CHOI 0.51 ±0.48 kg; $F_{3, 28} = 12.00$; P<0.01), equating to PLAR 1.87 ±0.61 and CHOR 1.76 ±0.39 % than the ingestion trials PLAI 0.60 ±0.31and CHOI 0.72 ±0.69 % of relative weight (P<0.01; Figure 5.12).

Percentage of relative weight loss between treatments showed a main treatment effect ($F_{3, 28} = 12.28$; P<0.001). Subjects consumed a mean total of 0.52 ±0.04 L in PLAI and 0.53 ±0.05 L in CHOI. Thus the relative sweat loss between the two ingestion treatments PLAI (0.94 ±0.22 L) and CHOI (1.04 ±0.29 L) were similar.

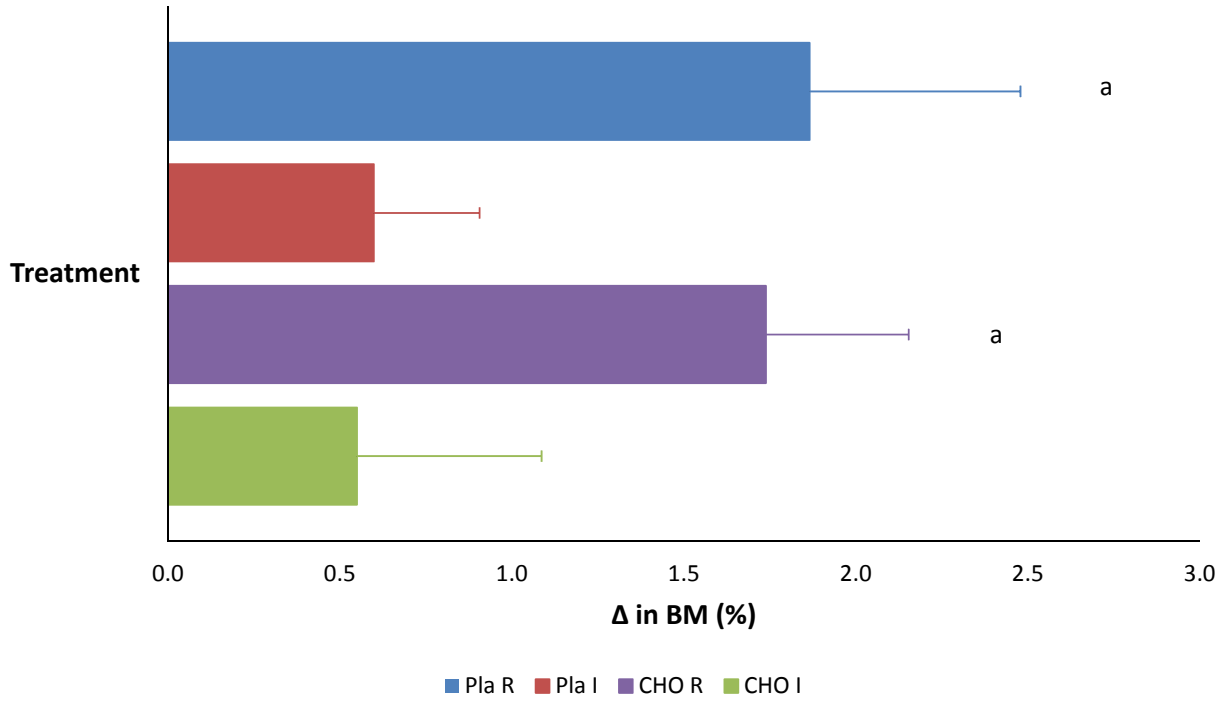

Figure 5.12 Change in body mass (%) during trials (mean ±SD; n = 8). (**a** = significantly different from PLAI and CHOI; P<0.001)

Figure 5.13 shows the mean HR data over the four trials. There was a significant time effect ($F_{3, 21}$= 1.26; P<0.05) where HR steadily increased from the start of exercise (0%; 87.3 ±6.8 beat·min^{-1}) to the end of exercise (100%; 120.0 – 166.6 beat·min^{-1}) in all four trials. There were no main treatment effects (P>0.05) or interaction differences (P>0.05).

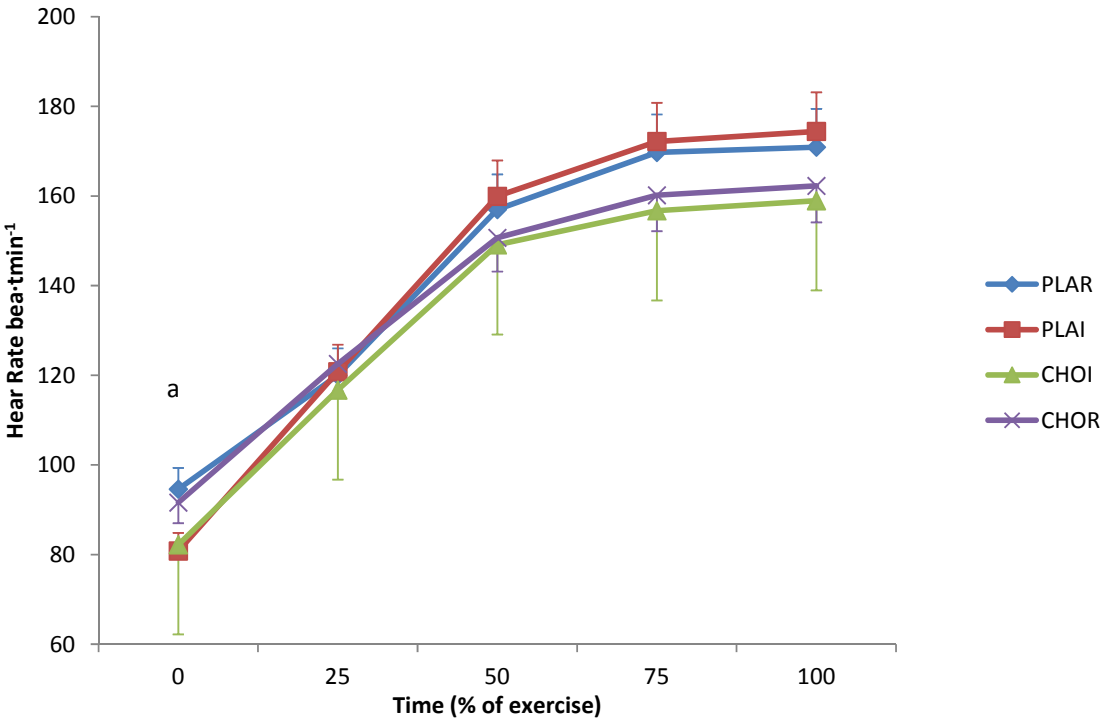

Figure 5.13 Mean (±SD) heart rate (HR) taken every 25% of exercise (n = 8). (Significant time effect of time: **a** = from rest of exercise to 100%; P<0.05)

5.7 Ambient temperature, humidity and barometric pressure.

There were no significant differences between trials for mean dry bulb temperature, relative humidity and barometric pressure (n = 32). Mean dry bulb temperature increased steadily during the trials, from 18.4 ±0.22 to 20.21 ±0.17ºC at the end of the time trial. Relative humidity also increased steadily during the trials, from 46.4 ±1.24 to 48.59 ±1.42% at the end of the time trial and there were no significant differences between the trials (P>0.05). Barometric pressure was unchanged between trials (1010.0 ±12.56 to 1010.94 ±2.75 mb; P>0.05).

5.8 Dietary intake

There were no statistical differences between trials in constitutes of the participants' diet (Table 5.6).

Table 5.6 Summary of constitutes of the participants' diet (mean ±SD; n=8)

Dietary intake	Total energy intake (MJ)	Carbohydrate (g)	Fat (g)	Protein (g)
PLA R	13.88 ± 56.7	845.1 ±197	218.4 ±83.7	312.3 ±63.3
PLA I	12.71 ±5.8	693.5 ±203.7	234.6±102.7	299.9 ±78.9
CHO R	12.29 ±5.5	729.1 ±195.3	196.8 ±55.9	306.3 ±83.7
CHO I	11.84 ±5.4	705.3 ±178.0	189.7 ±40.0	284.7 ±76.4
Mean	**12.68±0.9**	**468.5±297.4**	**140.2±77.8**	**188.2±120.8**

6.0 Discussion

The present study set out to examine whether there were individual and/or additive effects of carbohydrate mouth rinse, fluid intake and carbohydrate ingestion on 1-h time trial cycling performance in a glycogen reduced, fasted state. One of the main findings was that there was no significant difference in performance time between the treatments however, when effect size was analysed the data suggested that there was indeed practical significance with a 4.6% difference between CHOI relative to the other trials (Cohen's d = 0.36-0.67). Power output was significantly different between treatments (P = 0.002) with post-hoc analysis showing CHOI to be higher than the other trials (P<0.05). With this in mind, according to the present data, in a fasted, glycogen reduced state, rinsing with a carbohydrate solution has no impact on performance. This suggests that the central effects associated with carbohydrate mouth rinsing (Carter et al 2004b, Jeukendrup et al 2004, Johnson et al 2006) may not be as evident in this study. The present study's results show similarities with previous observations that ingestion of a carbohydrate solution improves endurance performance in the fasted state when compared to mouth rinsing a carbohydrate solution (Rollo et al, 2011).

The project further investigated the response in circulating markers of fuel utilization and found that in the CHOI trial, glucose concentrations were significantly higher than the other three trials (Figure 5.4; P< 0.01), particularly from 75-100% of the time trial (P<0.05). The importance of blood glucose in providing a source of fuel for exercising muscle and nervous tissue is common knowledge (Costill et al, 1977; Jeukendrup et al,1997; Rollo et al, 2011) and the results in the present study have emphasised this. To the author's knowledge, the current study is the first to examine the central and peripheral effects of carbohydrate ingestion and mouth rinsing in cycling performance in the fasted state. Much of the evidence in the present study illustrates that in a cycling time trial with participants in a fasted state, carbohydrate ingestion appears to improve endurance performance by maintaining high rates of glycaemia and carbohydrate oxidation. The current study however, did not show any significant differences in carbohydrate oxidation. These findings are in contrast with Beelen et al (2009) who reported no improvements in cycling performance following carbohydrate mouth rinsing when participants were fed compared to a fasted state. In their study, participants were asked to complete a cycling time trial after consuming a standardised

breakfast with either a 6.4% maltodextrin mouth rinse solution or a placebo mouth rinse. Performance time, workload and RPE were similar between the two treatments at all the time points. Previous speculations regarding the mechanisms behind the carbohydrate mouth rinse's ergogenic effects observed in exercise suggest that upon stimulation of carbohydrate receptors in the mouth there are pleasure and reward centres in the brain that are stimulated (Carter et al 2004[b]). However, Beelen et al (2009) speculated that when liver glycogen stores are available and endogenous glucose levels are not compromised it seems there is an absence of ergogenic effects upon carbohydrate supplementation and performance. However, unlike the current research they failed to examine blood glucose in their participants which may have clarified their findings to some extent. They also suggested that further research should re-examine the effect carbohydrate supplementation has on mood and performance when glycogen stores are compromised and the current study explored this suggestion further.

In contrast with Beelen et al (2009), previous research has found that ingestion and mouth rinsing with carbohydrate immediately facilitates corticomotor output in both fatigued and fresh muscle and this occurred prior to the peripheral availability of glucose (Gant et al 2010). The authors suggested that the ergogenic effects experienced during exercise are likely due to the novel mechanism of sensorimotor integration and the activation of receptors in the mouth that may stimulate corticomotor output. Although there were immediate ergogenic effects observed in maximal voluntary force and motor evoked potentials with the presence of carbohydrate in the mouth, the authors also explained that with the peripheral appearance of blood glucose, there was an increase in force, however this had no effect on the primary motor cortex. It was suggested that the increase in force production that was observed with an increase in plasma glucose concentrations after carbohydrate ingestion was related to peripheral factors rather than central factors. The authors further speculated that increased glucose uptake and exogenous carbohydrate oxidation are likely to explain this, however, further analysis with the oxidation rates and the relative contribution of the central and peripheral effects on endurance performance was needed. In the current study, the participants underwent a cycling glycogen reduction protocol followed by an overnight fast (12 h) prior to the performance tests. The results seem to show that there was a lack of ergogenic effect in performance with carbohydrate mouth rinsing, suggesting that the postulated central effects surrounding the idea of mouth rinsing with a carbohydrate solution

are weak. Indeed, carbohydrate ingestion was shown to enhance parameters of performance and this may have corresponded with increases in plasma glucose concentrations which suggest that in a glycogen reduced, fasted state, there is an increased sparing of glycogen stores (Coyle et al, 1986). However, as muscle glycogen concentrations were not measured, further research is needed to support this speculation. There may also be better maintenance of blood glucose and thus increase in carbohydrate oxidation as suggested by the estimated rates of carbohydrate oxidation (Rollo et al, 2011). The results in the current study suggest that there are associated ergogenic effects on performance with carbohydrate ingestion and are likely of a peripheral nature.

The ergogenic effects on endurance exercise in the present study were represented by the performance findings. As reported earlier, there was a non-significant difference between the treatments in performance time in a 60 min high intensity time trial. More specifically, when the percentage differences between trials were examined, participants seemed to be 4.2-4.6% faster in the CHOI trial compared to the other trials. A priori sample size estimation suggested 10 subjects would be sufficient to show 5% changes in performance however, due to unforeseen circumstances, only 8 subjects completed the study. These results are similar to the 2.3% increase (~1 min faster) reported during a cycling 40-km time trial where a 6.5% carbohydrate solution was ingested compared to a placebo solution (Jeukendrup et al 1997). Although the results from Jeukendrup et al (1997) were statistically significant the percentage of improvement was marginal and a placebo solution was compared with the carbohydrate solution, whereas in the present study there were four trial solutions. Jeukendrup et al (1997) also did have a much larger sample size (n=19) compared to the present study. The suggested 4.2-4.6% decrease in performance time in the present study agrees with previous observations that ingestion of a carbohydrate solution can improve 1-h running performance in fasted runners (Rollo et al 2009).

The non-significant increase in performance time is a result of increases in power output – the other performance parameter (Figure 5.1). The participants started the time trial at 75% Wmax and were blinded from the power output value throughout the test; however, they were able to adjust the resistance during exercise. It was reported that mean power output was higher in the carbohydrate ingestion trial relative to the other trials (CHOI: 231.6 W; CHOR: 222.1 ±15.7 W; PLAR: 224.6 ±16.2 W and PLAI: 223.4 ±19.4 W; $P<0.001$). The question, as

to why there was a significant difference observed in power output compared to no significant differences in performance time data, could be a result of a smaller sample size. Similarly, Anataraman et al (1995) reported a non-significant 11% improvement in power output with a 10% glucose solution compared to a placebo solution during a 1-h high intensity cycling time trial. The authors only used five participants and suggested that, had there been a larger sample size, their results may have been statistically significant. Moreover, other research that have not observed significant differences when analysing performance effects may also be result of issues with statistical power (McConell et al, 2000; Nikolopoulos et al, 2004; Desbrow et al, 2004). If there had been a statistical significance difference in performance time, the present study's speculations that peripheral effects are indeed the main influence on endurance performance would have been further confirmed. Support for this contention comes from Desbrow et al (2004) who although showed no statistical significance in performance data, did show a trend towards the carbohydrate ingestion trial (6% CHO) compared to the placebo trial with performance improvements and suggested that there were also issues with statistical power.

Further analysis of the performance variables showed that there was a moderate, inverse correlation between performance time and average power output ($r = -0.405$; $P<0.05$). Therefore, other factors such as pacing strategy may have contributed to the weaker correlation (Foster et al, 1993). However, the current research was designed to negate this issue through a cross over design. Furthermore, Hagberg et al (1981) noted that the most efficient pedalling frequency was 91 rpm in a group of trained road cyclists. Speculations have risen that pacing strategy is a function of metabolic and biochemical afferents to the CNS that have developed from past experiences (Johnson, 2006). The participants in the present study were informed to cycle at 90 rpm during the trial and therefore other factors such as between participant differences in cycling technique may have been the reason for the moderate correlation results. Future research may wish to examine pedal frequency as a control measure.

A further reason for the disparity in the performance results may have been due to the variation in $\dot{V}O_2$peak data and the subjects' baseline training variability. Ideally, elite cyclists or triathletes would have been preferred due to the more consistent baseline training they would have compared to the training and activity of the recreational cyclists and triathletes

used in this study. It must also be noted that participants in an earlier study (Jeukendrup et al 1997) had a $\dot{V}O_2$peak of over 60 ml·kg^{-1}·min^{-1} whereas in the present study the participants had a mean $\dot{V}O_2$peak of 53.3 ml·kg^{-1}·min^{-1}. Although there was variability among the participants' baseline training levels, during the time trials the subjects were initially performing at a work rate of ~80% $\dot{V}O_{2peak}$ and this decreased to 76.8% $\dot{V}O_{2peak}$ at the end of the time trial, with a mean work rate of 78.55% $\dot{V}O_{2peak}$ and thus there was a main effect of time on $\dot{V}O_{2peak}$ (Table 5.3; P=0.05). This is similar to values recorded in other cycling time trials (Jeukendrup et al 2002). The decrease over time in power output and work rate in both the present study and other endurance performance studies therefore may be an accurate representation of the physiological demands experienced in actual endurance events such as cycling events (Faria, 2005).

In the present study glucose concentrations were significantly higher in the CHOI trial when compared to the other three trials (P<0.001). Increases in blood-borne glucose have been said to develop when there are sufficient liver glycogen stores and glycogen precursors available (Reynolds et al, 1985). Gant et al (2010) investigated the effects of carbohydrate on corticomotor excitability and voluntary force production and reported that there were significant main effects of treatment (P<0.001) and time (P<0.001) for plasma glucose ingestion in the carbohydrate ingestion trial. However, their results did not show that the increase in blood glucose increased corticomotor activity and had no effect on the primary motor cortex intracortical inhibition or twitch force. Although, there was an increase in voluntary force production upon the inflection of plasma glucose reported. In their carbohydrate mouth rinse trials there were no blood samples taken. Rollo et al (2011) did analyse blood glucose and lactate in their study. The authors reported no significant differences in blood glucose, blood lactate, oxygen uptake and heart rate during the time trial however, there was a significant difference in running performance (P=0.019) in the carbohydrate ingestion trial when compared to the carbohydrate mouth rinse trial and the placebo ingestion solution. The authors suggested that the lack of differences in the metabolic data may have been a result of the methods they used in monitoring the physiological changes during exercise i.e. they were not sensitive enough to detect the minor changes in running speed. During exercise they reported blood glucose concentrations in the carbohydrate ingestion trial between 4-5 mmol·L^{-1}. In the current study blood glucose concentrations were reported between 4.4-5.9 mmol·L^{-1} in CHOI with significantly higher plasma glucose concentrations compared to the CHOR, PLAR and PLAI trials and there was

a significant difference in performance in the CHOI trial compared to the other trials. The present study's data suggests that the brain seems to not be 'fooled' by the carbohydrate mouth rinse solution when the plasma glucose concentrations are significantly lower. As a result of the lack of central and peripheral effects in the carbohydrate mouth rinse trial there were no ergogenic effects on performance.

Investigations have in the past demonstrated that blood glucose concentrations may decrease during long-term exercise, thus suggesting that exhaustion in such exercise may be associated with the lack of available blood glucose (Costill et al, 1977). The present study is in agreement with Costill et al (1977) as there was a linear decrease (4.9-3.9 $mmol \cdot L^{-1}$) shown in blood glucose concentrations in the carbohydrate mouth rinse, placebo mouth rinse and placebo ingestion trials which corresponded with a decrease in performance when compared to the consistently higher glucose concentrations in the carbohydrate ingestion trial (4.4-5.9 $mmol \cdot L^{-1}$). The decrease in performance in the carbohydrate mouth rinse when compared to the carbohydrate ingestion trial may therefore be a result of lack of available blood glucose as the participants were in a glycogen reduced state prior to beginning the performance trials (Costill et al 1986; Tsintzas et al 1995).

Although it is still unclear, the increase in blood glucose observed in the carbohydrate ingestion trial may reflect the ability of exogenous carbohydrate in its support of a high carbohydrate oxidation when endogenous carbohydrate availability would be limiting (Coyle et al,1986). In the present study the participants were in a glycogen reduced state and it may seem obvious that the exogenous carbohydrate may have had an effect on blood glucose and carbohydrate oxidation, as exogenous carbohydrate provides an alternative substrate to compensate for the reduced endogenous carbohydrate availability (Jeukendrup, 2004). However, the estimated amount of exogenous and endogenous carbohydrate oxidised was not analysed. Thus, it may be interesting to model how much of the increase observed in glucose reflects exogenous carbohydrate or a change in glycogenolysis. There are a number of mechanisms as to why the carbohydrate ingestion may have improved endurance performance including the maintenance of glucose. Previous research also suggests that higher levels of carbohydrate oxidation are related to the improvement (Jeukendrup et al 2004, Coyle et al 1986, Mitchell et al 1989). Coyle et al (1986) found that fatigue occurred in the cycling trials that were not supplemented with carbohydrate when glucose levels were markedly depressed. It seems that in the present study endurance performance may be

sensitive to the relatively small declines in blood glucose. Another mechanism to explain the performance improvement is the speculation that carbohydrate ingestion is involved with the sparing of muscle glycogen (Nicholas et al, 2000; Tsintzas et al, 1995) Due to the muscle glycogen stores being reduced prior to the trials, it is unlikely that an increased rate of glycogenolysis could contribute a significant amount the non-significant increase in carbohydrate oxidation in the CHOI trial and this may reflect the contribution of the exogenous carbohydrate (Mitchell et al 1989).

Previous research has demonstrated that with the ingestion of carbohydrates, lipolysis was suppressed sufficiently to reduce fat oxidation and muscle glycogen oxidation was increased to maintain energy production during exercise (Hargreaves et al, 1988). It can be speculated that the exogenous CHO ingestion may lead to an improved maintenance of carbohydrate oxidation and enhanced performance (Faria, 2005; Pottier, 2008). With regards to the blood data, in the CHOI trial there were markedly higher concentrations of blood glucose which correspond with the non-significant increases seen in the carbohydrate oxidation data that was estimated from indirect calorimetry equations. It is tempting to speculate that the ingestion of the 7.5% carbohydrate solution may then have resulted in the sparing of glycogen, even in a fasted glycogen reduced state and the body utilised this carbohydrate and thus, an increase in performance (4.6%) was documented compared to CHOR where there was no ingestion of carbohydrate and no result on performance. Although the glycogen concentrations in the participants were not analysed in the present study, future research could scrutinise these findings further through means of muscle biopsy or use of isotopes to trace the glycogen. The fact that the participants were in a fasted state may also be criticised as not being realistic in the practical sense. However, the present findings in the fasted state may still prove to be an accurate representation of endurance performance as it is not uncommon for athletes who have early morning events to begin exercise in a fasted state, for example Ironman competitors (Rollo et al, 2011).

The increase in glucose concentrations in the CHOI trial corresponds with a significant interaction effect of insulin in the CHOI trial ($P<0.001$; Figure 5.5). Insulin is the principle hormone responsible for the control of glucose metabolism (Coggan et al, 1988). C-peptide (a marker of insulin secretion) also showed a significant treatment effect ($P=0.04$; Figure 5.6). Both insulin and C-peptide showed main effects of time ($P<0.01$) and decreased during

exercise. However, during the CHOI trial, insulin levels were higher than the other trials during 50% and 75% time points ($P<0.05$). In relation to performance it would be wise to question if the changes insulin at least in part would explain the increase in power output in the CHOI trial compared to the CHOR trial. The power output could very well be driven by changes in insulin sensitivity which drives fuel utilisation. These results, along with the plasma glucose increase in the CHOI trial, may suggest that there is indeed an increase in insulin sensitivity. Perhaps the exploration of change in insulin sensitivity and its effect on endurance performance could be analysed in further research. The change in insulin observed in the CHOI trial, particularly at 50% and 75% of exercise, may not reflect a difference in insulin secretion which is intriguing and perhaps further analysis of insulin sensitivity through homeostatic model assessments (HOMA) and dynamic insulin sensitivity tests (DIST) could scrutinise this speculation. HOMA insulin resistance (IR) and DIST analysis are methods of assessment of cell function and IR from basal (fasting) glucose and insulin or C-peptide concentrations (Wallace, 2004). C-peptide itself is a strong measure of insulin secretion but not of insulin action, and the concept of the model revolves around the fact that insulin sensitivity is a function of glucose metabolism driven by the action of insulin (Wallace, 2004). It may be feasible that other blood plasma parameters that reflect reduced insulin sensitivity (e.g. leptin, glucagon-like peptide-1 (GLP-1), interleukin-6 (IL-6), and ghrelin also be investigated in the future to clarify the current observations.

As the participants' power output was significantly higher in the CHOI trial ($P=0.002$), there was an interaction of treatment and time in blood lactate concentration where lactate was higher in the CHOI trial at 100% ($P<0.05$; with concentrations up to 4mmol·L^{-1}; Figure 5.7), thus suggesting that there was a higher anaerobic glycolysis in this trial. The results in the present study are similar to Rollo et al (2011) who reported that in the carbohydrate ingestion trial, there were non-significant higher concentrations of blood lactate and carbohydrate oxidation. When compared to a 6.5% carbohydrate mouth rinse and a placebo mouth rinse, the participants significantly covered more distance ($P=0.019$) in the carbohydrate ingestion trial. Rollo et al (2011) recently examined the effects of a carbohydrate mouth rinse vs. carbohydrate ingestion in fasted states, and compared to the present study's methods there was a lack of metabolic and endocrinal data analysed to clarify their speculations. However, the present study's results confirm their observations with the ergogenic effect of a

carbohydrate solution on endurance performance in which Rollo et al (2011) explained to be a consequence of an increased rate of exogenous carbohydrate or elevated RER.

Further analysis of the expired air variables showed that there was a weak but significant correlation between the carbohydrate oxidation rates and mean power output ($r = 0.192$; $P=0.03$), and fat oxidation rates and mean power output ($r= 0.224$; $P=0.01$). Although there were no significant differences and there were weak correlations between the estimated energy expenditure rates and mean power output, practical significance was achieved with medium to large effect sizes between CHOI relative to the other trials. It has been suggested that when blood glucose levels are maintained the rate of glucose uptake and thus carbohydrate oxidation, can be high in well-trained endurance athletes (Coggan et al 1987). With this in mind, it may be likely that a large amount of the carbohydrate that was ingested was oxidised during the cycling time trial and this allowed the extramuscular carbohydrate stores to remain higher in the carbohydrate ingestion trial when compared to the carbohydrate mouth rinse and placebo trials (Coyle et al 1986). Previous research examining the effects of a carbohydrate mouth rinse on performance has postulated that there is a centrally-mediated impact on performance for example on muscle force generation (Gant et al 2010). The current study's results do not appear to show that there is an increase in carbohydrate oxidation in the carbohydrate mouth rinse relative to the placebo treatments and thus, may not be the reason why performance may increase. In the current study performance trials were undertaken in a glycogen reduced state, it is unlikely that an increased rate of glycogenolysis could have contributed a significant amount to the non-significant increase of carbohydrate oxidation in the carbohydrate ingestion trial. It appears that the exogenous carbohydrate resulted in an increase availability of blood glucose concentrations which increased the estimated carbohydrate oxidation rates.

There was no statistical significance reported with RER in the present study. The lack of significant RER results reported in Rollo et al (2011) along with the present data could be an effect of insufficient collection of the gas parameters. In the present study, the expired air samples were collected using the Douglas bag method every 20% of exercise, corresponding to four final samples. Perhaps had there been further collections the differences seen in the RER values in the CHOI trial may have been more prominent resulting in statistical significance. However, due to the nature of the test being a performance test, the researchers

felt that further sampling of expired air would have been detrimental to the participant's performance. Hence, there were also no significant differences found in estimated CHO expenditure rates (P=0.09) and estimated fat expenditure rates (P=0.29) between treatments even though there seem to have been higher rates of carbohydrate oxidation and lower rates of fat oxidation in the CHOI trial compared to the other three trials. However, emphasis must be given on the indirect calorimetry equations used to estimate the energy expenditure rates as the calculations are based on several assumptions including that large between-participants difference are probable (Bangsbo, 1994).

Losses of 2% body weight during exercise have been reported to decrease endurance performance by 10% (Saltin et al 1988). In the present study, the participants' mean relative body mass loss was greater in the two mouth rinse trials compared to the two ingestion trials (P<0.01; Table 5.7). There were also non-significant decreases in plasma volume relative to pre- exercise plasma volumes during the mouth rinse trials and this may coincide with the change in body mass reported in the mouth rinse trials. If fluid loss had been prominent in the present study, dehydration would have increased glycogenolysis (Haregreaves et al, 1996), there would have been a reduced central drive to exercise (Saboisky et al, 2003), an increase in cardiovascular function (increased heart rate, plasma volume loss), resulting in an increase in physical strain (Hamilton et al, 1991). As a result performance should have been better in both the PLAI trial and the CHOI trial relative to the mouth rinse trials. However, the PLAI trial did not manifest any ergogenic effect on performance relative to the rinse trials thus suggesting that fluid loss and/or dehydration are not the underlying cause of fatigue in the present study. In the present study fluid was administered in the ingestion trials at a rate of 1.5 $ml \cdot kg^{-1}$ BM every 12.5% of exercise but only ~40% of the corrected sweat loss was replaced using this strategy. Rollo et al (2011) used 2 $ml \cdot kg^{-1}$ BM solution in their ingestion trial and found a significant 2.2% difference in performance in the carbohydrate ingestion trial compared to the carbohydrate and placebo mouth rinse trials. This suggests that there was greater fluid replacement to replenish all body water loss and thus may be the reason behind the difference between the current study and their findings. In the current study there was statistical significance with CHOI in the power output data corresponding to a 4.6% decrease in performance time, this equates to a 3 min difference when compared to the other trials (CHOR, PLAI, PLAR). It is tempting to speculate that the increase in performance is not simply a result of hydration but indeed is related to the amount of carbohydrate in the solution also.

From the POMS data there were non-significant reduced self-reported perceptions of fatigue (Figure 5.9) and vigour (Figure 5.10) reported in the carbohydrate ingestion trial. The current study is the first to examine the effects of mood and performance with carbohydrate ingestion and mouth rinsing in the fasted state. Previous research has speculated that there are central effects associated with carbohydrate mouth rinsing on exercise performance, where it has been hypothesised that upon the presence of carbohydrate in the mouth, pleasure and reward centres of the brain are stimulated and that ergogenic effects on performance are a result of the stimulation of these pleasure centres (Gant et al 2010; Pottier et al 2008; Rollo et al 2010). In the present study both the perceptual effects of mouth rinsing and ingestion with a carbohydrate solution were analysed during a cycling time trial and it may be assumed that if there were indeed central effects present in the carbohydrate mouth rinse trial, there would also be perceptual effects observed in carbohydrate ingestion. Moreover, the current research has shown no significant differences in perceptual data in the CHOR trial however, in the CHOI trial, there seems to be a trend relating to increased reports of vigour and decreases in fatigue. This may suggest that a different mechanism may be present than the previous speculations that certain areas of the brain are believed to be stimulated by carbohydrate (anterior insula, striatum) and that these particular areas may not be responsible for mood (Haase et al, 2009; Liu et al, 2000).

Previous research has specified that ingestion of a carbohydrate-electrolyte solution has resulted in more positive mood states during prolonged cycling and lower negative mood scores of fatigue, depression and tension (Keith, 1991; Lieberman, 2002). The results of the current study show similarities with the findings of Backhouse et al (2007) suggesting that ingestion of a carbohydrate-electrolyte solution might induce a ‘feel good’ effect during exercise. An impaired function of the CNS has also been linked with accumulation of 5-HT in the brain during exercise which is believed to be a result of higher FFA concentrations in the blood resulting in an increase of tryptophan transport to the brain where it is then converted to 5-HT (Winnick, 2005). As observed in the current study, increases of FFA in the CHOR, PLAR and PLAI trials may have resulted in increases in feelings of fatigue and lower vigour feelings. It seems that ingestion of a carbohydrate solution during the cycling time trial could have reduced ammonia production and lowered 5-HT accumulation in the brain and thus reduced the dopamine inhibitory effects of 5-HT (Winnick, 2005). This corresponds with the POMS data in the present study with non-significant decreases in fatigue and

increases on positive mood states such as vigour reported (Winnick, 2005). However, in the present study 5-HT and tryptophan levels were not measured and these speculations could be revisited in further research. Future research re-examining the 'pleasure centres' associated by the presence of carbohydrate may also be explored.

Backhouse et al (2005) reported that when carbohydrate was ingested during prolonged cycling, throughout the exercise, pleasure-displeasure was enhanced and the effect of perceived exertion was limited. Ali et al (2007) reported that during intermittent exercise, RPE was lower in the carbohydrate ingestion trial when compared to a placebo solution and this subsequently resulted in increased sprint performance. In the current research there were no significant differences in RPE between trials suggesting that participants perceived to be performing just as hard in all of the trials. However, on examination we see improved performance effects in the CHOI trial, therefore the use of an ingestible carbohydrate solution can improve endurance performance without significant increases in perceived exertion.

The current findings suggest that there is a lack of ergogenic effects on performance with carbohydrate mouth rinsing. Pottier et al (2008) reported improved performance in the carbohydrate mouth rinse trial when compared to the carbohydrate ingestion and placebo trials. However, the participants in the study were not blinded by the treatments and may have been aware of the speculations regarding carbohydrate mouth rinsing and performance and this may have to some extent had an effect on the results. Other studies have not found ergogenic effects with 5 s mouth rinse protocols (Whitham et al (2007); however in this study the subject sample size was low (n=7) and statistical power may be questioned. On the other hand, Just et al (2008) reported a release of insulin in response to glucose in the mouth when participants held the carbohydrate solution in their mouth for 45 s. However, the amount of swill post-expectoration was not measured and it can be speculated that some of the solution may have been swallowed. Nevertheless, this is only a speculation and perhaps the longer period of mouth rinsing may indeed be appropriate in order to see the ergogenic effects. In the current study there were no documented increases in insulin in the CHOR trial. The protocol used in Just et al (2008) may be arguably too long and unrealistic to use in a practical sense in a sports or exercise environment. Further research could examine the duration of swilling on various aspects of metabolism, perception and performance.

Previous research with carbohydrate mouth rinsing have used solutions with 6.5% carbohydrate (Beelen et al 2009; Rollo et al 2011; Whitham et al 2007) and have reported no effects on performance. On the other hand, there have also been studies that have used similar amounts (6-6.4%) and have reported improvements in performance in the carbohydrate mouth rinse trial (Carter et al 2004b, Pottier et al 2008; Rollo et al 2010). The present study used a 15% carbohydrate mouth rinse solution and still found no ergogenic effect on performance and the discrepancies in previous studies may be a result of swill time and/or the weighed expectorate rather than amount of carbohydrate in the mouth rinse solution.

Ingestion of carbohydrates during exercise has been known to cause gastrointestinal (GI) discomfort (van Nieuwenhoven et al, 2005). GI discomfort during exercise has also brought about decreases in performance (van Nieuwenhoven et al, 2005). High oxidation efficiency is when there are smaller amounts of carbohydrate that remain in the gastrointestinal tract thus reducing the risk of GI discomfort (Jeukendrup, 2004). Multi transportable carbohydrate solutions such as the solution (maltodextrin-sucrose) used in the current study have been reported to reduce the likelihood of GI distress (Jeukendrup, 2004). Although GI discomfort was not assessed in the present study, when the participants were asked if they had experienced GI discomfort there were no complaints. Anecdotal comments suggest that many of the participants felt thirsty and dehydrated during the rinsing trials and thus preferred the ingestion trials. The participants also reported that they were very tempted to swallow the solution because of the increase in thirst. The participants also reported that it was difficult to hold their breath while they swirled the solution around their mouth and they found it hard to maintain their cadence for the 8 s during the mouth rinse treatments. Issues relating to social acceptability may also deter athletes from pursuing such a method. Due to the issues of hygiene and the practical aspects, further analysis is needed to examine if sports performance mouth rinsing is possible in a ‘real life’ sporting situation. Other than if an athlete experiences GI distress, there seems to be no other reason why one may not be able swallow a solution.

6.1 Summary

The present study set out to examine whether there were individual and/or additive effects of carbohydrate mouth rinse, fluid intake and carbohydrate ingestion on 1-h time trial cycling performance in a glycogen reduced, fasted state. During moderate-high intensity exercise of

~60 min duration previous research has suggested that there is sufficient glycogen to supply energy over this period of time (Below, 1995; Pottier, 2008). With this in mind, according to the present data, in a fasted, glycogen reduced state, rinsing with a carbohydrate solution has no impact on performance. This suggests that the central effects associated with carbohydrate mouth rinsing may not be as evident in this study.

The results confirm previous findings that ingestion of a 7.5% carbohydrate solution improved endurance performance in the fasted state (Rollo et al, 2009; Rollo et al, 2011). It is tempting to speculate that the mechanism responsible for the improved performance in the carbohydrate ingestion trial is likely due to an increased rate of exogenous carbohydrate oxidation as a result of increased levels of glucose (Figure 5.4). These performance benefits have shown a delay in fatigue and demonstrated a greater ability to maintain a higher power output over time ascribed to increases in blood glucose and insulin concentrations. Other high intensity exercise studies have also found similar results (Winnick et al 2005). It has been well documented that carbohydrate ingestion during exercise can improve exercise performance and increase exercise capacity (Jeukendrup et al, 2008; Jeukendrup et al, 2010; Jeukendrup, 2004).

The project further investigated the response in circulating markers of fuel utilization between CHO ingestion and CHO rinse trials. The plasma glucose response in the CHOR trial was similar to the PLAR and PLAI trials however there was maintenance of blood glucose in the CHOI trial. Moreover, the increased blood glucose after 75% of exercise corresponded with higher power output in the CHOI trial. This suggests that there are indeed peripheral ergogenic effects in endurance performance associated with carbohydrate ingestion when compared to the lack of performance and associated central effects in carbohydrate mouth rinsing. Even though the participants had an increased mean power output in the CHOI trial (treatment main effect P=0.002) resulting in a 4.6% non-significant decrease in performance time, data shows that through mood assessment the participants found the exercise to be less fatiguing in the CHOI trial and showed corresponding increase in vigour emotions in the CHOI trial compared to the three other trials. In contrast with previous research, the current study's protocol allowed the researchers to analyse such parameters and their relation to one another (performance, metabolic, endocrinal, perceptual) in the same protocol, so as to examine the central and peripheral effects of carbohydrate mouth rinse, fluid intake and carbohydrate ingestion on 1-h time trial cycling performance in a glycogen reduced, fasted state.

6.3 Experimental Limitations

- A priori analyses suggested a sample size of 10 subjects would be required to examine performance changes between trials. However, due to unforeseen circumstances, only data from 8 participants were reported and thus at times statistical power was limited.

- Although the current study was double blind for CHO content, it was not possible to keep hidden from the participants whether a mouth rinse or ingestion solution was being used and therefore there may have been a small chance of bias between the trials.

- There was a small amount of variability in participant $\dot{V}O_{2peak}$ scores and subject baseline training. Preference for elite triathletes/cyclists would have been ideal as $\dot{V}O_{2peak}$ scores and training may have been more consistent.

- Although the participants were asked to maintain a cadence of 90 rpm (Jeukendrup et al, 1997), there may have been different pacing strategies utilised by the participants. Therefore, future studies may wish to examine aspects of cadence control / pacing strategies during this performance test.

6.4 Recommendations for future research

- Examination of insulin sensitivity by methods of HOMA IR and/or DIST analysis may be warranted in future research to examine how much influence insulin sensitivity has on endurance performance.

- To further investigate the central factors associated with performance improvement and carbohydrate sensing. This could be administered through measurement of variables such as 5-HT, tryptophan, dopamine, adrenalin response to carbohydrate mouth rinsing vs. carbohydrate ingestion on performance. Future analysis may

emphasise ‘central effects’ findings that have been reported in other studies with carbohydrate supplementation.

- GI discomfort has been closely associated with carbohydrate ingestion and its presence has been associated with detrimental effects on performance. Further research should consider using a GI discomfort questionnaire.

- Further research could model how much of the increase in blood glucose reported reflects exogenous carbohydrate compared to endogenous carbohydrate. This could be explored further through tracer studies with labelled carbohydrate isotopes.

- Further research may examine the amount of time a carbohydrate mouth rinse solution is ‘swilled’ as there have been many discrepancies with carbohydrate mouth rinsing on exercise performance and this may relate to the amount of time the solution in question is present in the mouth.

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8.0 Appendices

Appendix 1: Participant information sheet

Appendix 2: Participant consent form

Appendix 3: Health Screening Questionnaire

Appendix 4: Shortened Profile of Mood States (POMS) questionnaire

Appendix 5: Feeling Scale (FS)

Appendix 6: Felt Arousal Scale (FAS)

Appendix 7: Borg's Rating of Perceived Exertion (RPE)

Appendix 8: Participant calibration sheet

Appendix 9: Nutrition diary

Appendix 10: Activity training log

Appendix 1

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The effect of mouth rinsing and ingestion of carbohydrate solution on short intensive cycling exercise and measure of changes in peripheral metabolic regulation and fuel utilisation

PARTICIPANT INFORMATION SHEET

Invitation to Participate in Research Study

We, Drs Ali, Yoo and Breier, and Ms Moss are Sport & Exercise Science and Human Nutrition researchers at Massey University, and are currently conducting a series of novel research studies with cyclists.

There is evidence that regular carbohydrate (CHO) and fluid ingestion during exercise can help maintain performance. However, for some athletes, ingestion of such solutions during high intensity exercise may be uncomfortable due to feelings of gut fullness (and possibly reduce performance). Carter et al (2005) showed that 1 h cycling time trial performance was improved when participants simply rinsed their mouths with CHO (without actually ingesting any of the fluid). Further, Gant et al (2006) showed that self-selected treadmill running pace was higher when a CHO mouth rinse regime was used. Nevertheless, whether this effect triggers the release of extra energy stored in muscles and/or liver remains to be seen. Therefore, the aim of this study is to investigate whether mouth rinsing with a CHO solution during the fasted state may trigger the release of extra energy stored in muscles and/or liver during or after short intensive cycling exercise. A further aim is to examine whether there are individual and/or additive effects of CHO mouth rinse, fluid intake and carbohydrate ingestion on 1-h time trial cycling performance.

Participant Recruitment

All participants (male cyclists/triathletes, aged 18-50 and in regular training) will be recruited from within the Greater Auckland area. Smokers, or those taking prescription or recreational drugs are excluded from this study. Approximately 10 participants will be recruited in order to give statistical significance to any findings. From participation in this study you will learn more about your fitness levels when performing short intensive cycling activity. You will be reimbursed for travel expenses with MTA vouchers and also receive a summary sheet containing the main findings of the study.

Risks/Discomforts of the study include:

- Feeling fatigued following cycling exercise
- Mild soreness from blood sampling
- Mild dehydration during exercise

Project Procedures and Participant Involvement

Before taking part in this study you will also be asked to complete a Heath Screening Questionnaire relating to health status, prior medical issues, and medications taken. This screening questionnaire is used to ascertain information that may conflict with the study, (i.e. if you are taking any medications that may interfere with the outcome of the study), and may ultimately prohibit you from participating. If you have any medical condition listed in the Health Screening Questionnaire, then we will have to exclude you from taking part. The information obtained on all study questionnaires is strictly confidential and will be used for the purposes of the present study only.

Part A: The first preliminary session will include a maximal oxygen uptake (VO_2 max) test to determine your fitness level, and you will also be asked to familiarise yourself with the 1-hr cycling time trial protocol. In the second session you will perform 90 min of cycling exercise followed by a low CHO meal and overnight fast.

Part B: You will be asked to come to the Laboratory for four main trials; each main trial takes place over two days. On Day 1 you will perform 90 min of cycling exercise followed by a low CHO meal and overnight fast. On Day 2 you will be asked to perform 1-h cycling time trial exercise under four separate conditions:

- A - Carbohydrate mouth rinse (after every 12.5% of performance ride)
- B - Placebo mouth rinse (after every 12.5% of performance ride)
- C - Carbohydrate ingestion (2 $ml \cdot kg^{-1}$ body mass after every 12.5% of performance ride)
- D - Placebo ingestion (2 $ml \cdot kg^{-1}$ body mass after every 12.5% of performance ride)

A 15% carbohydrate solution will be used for the rinsing trial (Trial A) whereas a 6-8% carbohydrate solution will be used for the ingestion trial (Trial C). Taste and colour-matched solutions containing 0% carbohydrate and artificial sweeteners (aspartame and/or acesulfame K) will be used for the other trials (Trials B and D). All solutions will be prepared according to Goods Manufacturing Practices that comply with Food Safety Australia and New Zealand regulations.

During your 1-h cycling time trial exercise, heart rate and oxygen uptake will be monitored at regular intervals. Ten millilitre blood samples will be collected, using venepuncture, before the cycling exercise (on Day 1) and via an indwelling catheter, before (1 x 10-ml sample), during (5 x 10-ml samples) and after (2 x 10-ml samples) the 1-h time trial (on Day 2); these will be stored for later analysis of metabolic and physiological variables. Various scales (Profile of Mood States or POMS; Felt Arousal Scale or FAS and Feeling Scale or FS) will be used to assess your perceptual state during and following exercise.

You will be asked to keep a food diary two days prior to the first trial, then follow the same dietary intake on days prior to the successive trials. You will be asked to refrain from alcohol, caffeine, tobacco and other exercise in the 48-h period before the main performance trials.

Individuals trained in resuscitation (NZ Red Cross First Aid, Level 2) and use of a defibrillator will be present for all exercise sessions. First aid kits are available on site and a defibrillator (if required) can be made available (following a telephone call to Campus Security) if needed. In addition, the researchers will be constantly monitoring physiological and perceptual variables that will aid in identifying any major issues.

Participant's Rights

You are under no obligation to accept this invitation. Should you choose to participate, you have the right to;

- decline to answer any particular question
- withdraw from the study at any time, even after signing a consent form (if you choose to withdraw you cannot withdraw your data from the analysis after the data collection has been completed)
- ask any questions about the study at any time during participation
- provide information on the understanding that your name will not be used unless you give permission to the researcher

- be given access to a summary of their personal data as well as project findings when it is concluded (in the form of a summary sheet)

Confidentiality

All data collected will be used solely for research purposes and has the possibility of being presented in a professional journal. All personal information will be kept confidential by assigning numbers to each participant. No names will be visible on any papers on which you provide information. If you are a student of one of the research team please note that your academic grades will not be affected whether you decide to complete the study or withdraw at a later time. All data/information will be dealt with in confidentiality and will be stored in a secure location for ten years on the Massey University Albany campus. After this time it will be disposed of by an appropriate staff member from the Sport and Exercise Science department.

Project Contacts

If you have any questions regarding this study, please do not hesitate to contact either of the following people for assistance:

Dr. Ajmol Ali (Sport and Exercise Science, IFNHH, Massey University)

(09) 414 0800 ext.41184; a.ali@massey.ac.nz

Dr. Michelle Ji Yeon Yoo (Human Nutrition, IFNHH, Massey University)

(09) 414 0800 ext.41297; j.y.yoo@massey.ac.nz

Prof. Bernhard Breier (Human Nutrition, IFNHH, Massey University)

(09) 373 7599 ext. 41185; b.breier@massey.ac.nz

Ms Catherine Moss (Sport and Exercise Science/Human Nutrition, IFNHH, Massey University)

021 299 2285; catherinemoss@hotmail.com

Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 10/01. If you have any concerns about the conduct of this research, please contact Professor Julie Boddy, Chair, Massey University Human Ethics Committee: Southern A telephone 06 350 5799 x 2541, email humanethicsoutha@massey.ac.nz

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Injury Prevention, Rehabilitation and Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury. If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Appendix 2

Massey University
COLLEGE OF SCIENCES

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The effect of carbohydrate mouth rinse on short intensive cycling exercise

CONSENT FORM FOR STUDY VOLUNTEERS

This consent form will be held for a minimum period of five (5) years

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I understand that I have the right to withdraw from the study at any time and to decline to answer any particular questions.

I agree to provide information to the researcher on the understanding that my name will not be used without my permission. (The information will be used only for this research and publications arising from this research project).

I agree to participate in this study under the conditions set out in the Information Sheet.

Signature: _____

Date: _____

Full Name (printed)

_____

Phone Number_____ **Age** _____ **Date of Birth** _____

Address_____

Participant code ☐

Appendix 3

Pre-Exercise Health Screening Questionnaire

Name:_____

Address: _____

Phone: _____

Age: _____

Please read the following questions carefully. If you have any difficulty, please advise the medical practitioner, nurse or exercise specialist who is conducting the exercise test. Please note that smokers and/or those taking prescription or recreational drugs are excluded from this study.

Please answer all of the following questions by ticking only one box for each question:

This questionnaire has been designed to identify the small number of persons (15-69 years of age) for whom physical activity might be inappropriate. The questions are based upon the Physical Activity Readiness Questionnaire (PAR-Q), originally devised by the British Columbia Dept of Health (Canada), as revised by [1]Thomas *et al.* (1992) and [2]Cardinal *et al.* (1996), and with added requirements of the Massey University Human Ethics Committee. The information provided by you on this form will be treated with the strictest confidentiality.

Qu 1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

Yes ☐ No ☐

Qu 2. Do you feel a pain in your chest when you do physical activity?

Yes ☐ No ☐

Qu 3. In the past month have you had chest pain when you were not doing physical activity?

Yes ☐ No ☐

Qu 4. Do you lose your balance because of dizziness or do you ever lose consciousness?

Yes ☐ No ☐

Qu 5. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?

Yes ☐ No ☐

Qu 6. Do you have a bone or joint problem that could be made worse by vigorous exercise?

Yes ☐ No ☐

Qu 7. Do you know of any other reason why you should not do physical activity?

Yes ☐ No ☐

Qu 8. Have any immediate family had heart problems prior to the age of 60?

Yes ☐ No ☐

Qu 9. Have you been hospitalised recently?

Yes ☐ No ☐

Qu 10. Do you have any infectious disease that may be transmitted in blood?

Yes ☐ No ☐

Qu 11. This test may include the taking of blood for glucose testing. Do you have any objection to this?

Yes ☐ No ☐

Qu 12. Have you ever suffered from any sleep disorders?

Yes ☐ No ☐

Qu 13. Do you have any allergies to skin preparations (e.g. alcohol swabs), adhesives (e.g. plasters) or dressings (e.g. bandages)

Yes ☐ No ☐

You should be aware that even amongst healthy persons who undertake regular physical activity there is a risk of sudden death during exercise. Though extremely rare, such cases can occur in people with an undiagnosed heart condition. If you have any reason to suspect that you may have a heart condition that will put you at risk during exercise, you should seek advice from a medical practitioner before undertaking an exercise test.

I have read, understood and completed this questionnaire.

Signature: _____ Date: _____

References

1. Thomas S, Reading J and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci* 17(4): 338-345.
2. Cardinal BJ, Esters J and Cardinal MK. Evaluation of the revised physical activity readiness questionnaire in older adults. *Med Sci Sports Exerc* 28(4): 468-472

Institute of Food, Nutrition and Human Health
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Auckland

Profile of Mood States (POMS)

PSYCHOMETRIC SCALE

SUBJECT ID _____ BLOCK/BEVERAGE _____

DATE _____ TIME _____ DAY _____

PROFILE OF MOOD STATES-SHORT FORM (POMS-40)

Refer to the definitions below. Consider how you are feeling right now, when CIRCLING the appropriate response beside each item. Please check to make sure you have responded to all the items.

FATIGUE	NOT AT ALL	A LITTLE	MODERATELY	QUITE A BIT	EXTREMELY
Worn Out	0	1	2	3	4
Weary	0	1	2	3	4
Bushed	0	1	2	3	4
Fatigued	0	1	2	3	4
Exhausted	0	1	2	3	4

ANGER	NOT AT ALL	A LITTLE	MODERATELY	QUITE A BIT	EXTREMELY	
Peeved	0	1	2	3	4	
Bitter	0	1	2	3	4	
Resentful	0	1	2	3	4	
Grouchy	0	1	2	3	4	Angry
	0	1	2	3	4	
Furious	0	1	2	3	4	
Annoyed	0	1	2	3	4	

VIGOR	NOT AT ALL	A LITTLE	MODERATELY	QUITE A BIT	EXTREMELY
Cheerful	0	1	2	3	4
Powerful	0	1	2	3	4
Full of Pep	0	1	2	3	4
Active	0	1	2	3	4
Energetic	0	1	2	3	4
Lively	0	1	2	3	4

TENSION	NOT AT ALL	A LITTLE	MODERATELY	QUITE A BIT	EXTREMELY
Restless	0	1	2	3	4
Nervous	0	1	2	3	4
On-edge	0	1	2	3	4
Tense	0	1	2	3	4
Uneasy	0	1	2	3	4
Anxious	0	1	2	3	4

ESTEEM	NOT AT ALL	A LITTLE	MODERATELY	QUITE A BIT	EXTREMELY
Embarrassed	0	1	2	3	4
Ashamed	0	1	2	3	4
Proud	0	1	2	3	4
Competent	0	1	2	3	4
Satisfied	0	1	2	3	4

CONFUSION	NOT AT ALL	A LITTLE	MODERATELY	QUITE A BIT	EXTREMELY
Bewildered	0	1	2	3	4
Forgetful	0	1	2	3	4
Confused	0	1	2	3	4
Unable to concentrate	0	1	2	3	4
Uncertain about things	0	1	2	3	4

DEPRESSION	NOT AT ALL	A LITTLE	MODERATELY	QUITE A BIT	EXTREMELY
Hopeless	0	1	2	3	4
Helpless	0	1	2	3	4
Sad	0	1	2	3	4
Worthless	0	1	2	3	4
Miserable	0	1	2	3	4
Discouraged	0	1	2	3	4

Grove, J.R., Prapavessis, H. Preliminary evidence for the reliability and validity of an abbreviated Profile of Mood States. *International Journal of Sport Psychology*. 1992 Apr-Jun Vol 23(2) 93-109.
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Morgan WP, Brown DR, Raglin JS, O'Connor PJ, and Ellickson KA. Psychological monitoring of overtraining and staleness. Br J Sports Med 21: 107–114, 1987.

Appendix 5

FEELING SCALE

+5	Very good
+4	
+3	Good
+2	
+1	Fairly good
0	Neutral
-1	Fairly bad
-2	
-3	Bad
-4	
-5	Very bad

Appendix 6

FELT AROUSAL SCALE

1 Low arousal

2

3

4

5

6 High arousal

Appendix 7

Borg's RPE SCALE

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

Appendix 8

Participant Calibration Form

Participant's name:_____
Age:_____
Vegetarian: **YES** **NO**
Height (m):_____
Weight (kg):_____
Seat height:_____
Wmax (Watts):_____
VO2max (ml.kg.min^{-1}):_____
MaxHR (bpm):_____
Pedalling rate for glycogen ex:_____
75% Wmax:_____
Total work (J):_____
=0.75 x Wmax x 3600

GAS

Familiarisation

Gas	1	2	3	4
Minute				
Collection Time (s)				
FeO_2 (%)				
$FeCO_C$ (%)				
Volume Sample for CO_2 analysis				
Volume Sample for O_2 analysis				
Volume in bag (l)				
Temperature expired air				

Trial 1

Gas	1	2	3	4
Minute				
Collection Time (s)				
FeO_2 (%)				
$FeCO_2$ (%)				
Volume Sample for CO_2 analysis				
Volume Sample for O_2 analysis				
Volume in bag (l)				
Temperature expired air				

Trial 2

Gas	1	2	3	4
Minute				
Collection Time (s)				
FeO_2 (%)				
$FeCO_2$ (%)				
Volume Sample for CO_2 analysis				
Volume Sample for O_2 analysis				
Volume in bag (l)				
Temperature expired air				

Trial 3

Gas	1	2	3	4
Minute				
Collection Time (s)				
FeO_2 (%)				
$FeCO_2$ (%)				
Volume Sample for CO_2 analysis				
Volume Sample for O_2 analysis				
Volume in bag (l)				
Temperature expired air				

Trial 4

Gas	1	2	3	4
Minute				
Collection Time (s)				
FeO_2 (%)				
$FeCO_2$ (%)				
Volume Sample for CO_2 analysis				
Volume Sample for O_2 analysis				
Volume in bag (l)				
Temperature expired air				

Appendix 9

The effect of carbohydrate mouth rinse on short intensive cycling exercise

PARTICIPANT NUTRITIONAL ANALYSIS FORM

Please write down all foods and drinks consumed for two days prior to participating in your first trial. This includes snacks, lollies, water, vitamins, supplements, etc. Be as specific as possible. You will be asked to eat the exact foods that you write down on this sheet on the two days prior to your second trial at approximately the same times.

• Please refrain from consuming foods/drinks high in caffeine (e.g. coffee, tea, soda).
• Please refrain from consuming alcohol.
• Please do not consume any food 2 hours before your main trial. You will be allowed to drink as much water as you like but not sports drinks or any other food
• Please bring this form with you on the day of your second trial.

Use this example as a guide:

Time	Description of Food/Drink Consumed	Amount
Breakfast		
7:30am	Eggs – scrambled w/ salt and pepper	2 eggs
	Toast – wheat with butter	2 slices
	Orange juice	1 glass
	Banana	1 large
	Multivitamin	1
Lunch		
12:30pm	Sushi take away from Tokyo – chicken teriyaki	6
	Water	2 glasses
Dinner		
6:00pm	Grilled chicken breast	1 breast
	Steamed vegetables (carrots, broccoli, capsicum)	1 cup
	Rice	1.5 cups (cooked)
	Milk	1.5 (tall) glasses
Snacks/Other		
11:00am	Yogurt – blueberry	1 cup
3:00pm	Pretzels	small handful
3:00pm	V drink	1 can

DAY 1 (two days prior to your main trial)

Time	Description of Food/Drink Consumed	Amount
Breakfast		
Lunch		
Dinner		
Snacks/Other		

DAY 2 (one day prior to your main trial)

Time	Description of Food/Drink Consumed	Amount
Breakfast		
Lunch		
Dinner		
Snacks/Other		

Appendix 10

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Auckland

The effect of carbohydrate mouth rinse on short intensive cycling exercise

TRAINING LOG

Please record all of your exercise training 48 hours prior to coming in for the evening session. Include enough detail so that you can repeat each exercise session prior to your following week's evening sessions. The activity refers to the type of exercise, *for example weight training, running, cycling, etc.* The route indicates the course you cycled or ran over, *for example Albany to Riverhead.* There is room to record two exercise sessions per day, if needed. If you have races or competitions you must record them also. **You will be required to replicate your training schedule leading up to the second block of the study!**

Participant's name_____

Date	**Time**	**Duration** *(min)*	**Type**	**Discipline**	**Intensity**
				Endurance ☐ Intervals ☐ Strength ☐ Skill ☐	Light- Easy ☐ Light - Medium ☐ Medium - Moderate ☐ Medium - Heavy ☐ High-Heavy ☐
				Endurance ☐ Intervals ☐ Strength ☐ Skill ☐	Light- Easy ☐ Light - Medium ☐ Medium - Moderate ☐ Medium - Heavy ☐ High-Heavy ☐
				Endurance ☐ Intervals ☐ Strength ☐ ☐	Light- Easy ☐ Light - Medium ☐ Medium - Moderate ☐ Medium - Heavy ☐

				Skill	High-Heavy ☐
				Endurance ☐ Intervals ☐ Strength ☐ Skill ☐	Light- Easy ☐ Light - Medium ☐ Medium - Moderate ☐ Medium - Heavy ☐ High-Heavy ☐
				Endurance ☐ Intervals ☐ Strength ☐ Skill ☐	Light- Easy ☐ Light - Medium ☐ Medium - Moderate ☐ Medium - Heavy ☐ High-Heavy ☐
				Endurance ☐ Intervals ☐ Strength ☐ Skill ☐	Light- Easy ☐ Light - Medium ☐ Medium - Moderate ☐ Medium - Heavy ☐ High-Heavy ☐

Comments: