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The Effects of Nutritional Aids on Exercise Performance in a Fed State

Simon Devenney
Technological University Dublin, simon.devenney@tudublin.ie

The Effects of Nutritional Aids on Exercise Performance in a Fed State

A Thesis Presented For the Award of Masters by Research

by

Simon Devenney (B.Sc.)

Technological University Dublin

Department of Applied Science

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Dr. Kieran Collins
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DECLARATION

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Abstract

Previous research has shown that mouth rinsing with a carbohydrate solution (CMR) can improve cycling performance while caffeine supplementation (CAFF) can improve power output in bouts of repeated sprints in a low carbohydrate availability state. The aim of the current thesis was to identify the effects of nutritional, in the form of CMR, CAFF supplementation & CAFF rinse, on exercise performance while athletes were in a fed state. A total of 32 recreationally active males (aged 22 ± 3yrs, body mass 71.5 ± 10.1kg, stature 173.5 ± 7.6cm, skinfolds 75.6 ± 19.3mm) participated across three studies, looking at time trial performance, time to fatigue and repeated sprint performance. Heart-rate responses, blood lactate, rate of perceived exertion was monitored throughout all three studies, while time to completion, time to fatigue and power output were monitored in separate studies. Results indicate that CMR (6% or 16%) significantly improve time of completion for cycling TT performance (5-7% improvement). CMR with caffeine supplementation improves time to fatigue in interval running while a combination of CHO+CAFF rinse has a moderate effect for peak power production in repeated sprints when compared to placebo and control. These findings along with previous research suggest that the regular use of CMR can decrease the risk of gastrointestinal distress reported by athletes, meaning the data can be applicable to both athletes and coaches in a real-world setting. The ability for mouth-rinse to improve maximal exercise performance in the absence of fatigue further suggests a central mechanism.

List of Abbreviations

AIC,	anterior insular cortex;
CAFF,	caffeine;
CHO,	carbohydrate;
CMR,	carbohydrate mouth rinse;
CNS,	central nervous system;
CO2,	carbon dioxide;
CONT,	control;
CP,	centipoise;
E,	electrolytes;
ES,	effect size;
FFA,	free fatty acids;
FO,	frontal operculum;
fMRI,	functional magnetic resonance imaging;
GI,	gastrointestinal;
GLUT4,	Glucose Transport Type 4;
GLUT5,	Glucose Transport Type 5;
HIT,	high intensity training;
HR,	heart rate;
L,	litre;
KG,	kilogram;
MALT,	maltodextrin;
MG,	milligram;
MM,	millimetre;
MRI,	magnetic resonance imaging;
O2,	oxygen;
OBLA,	onset of blood lactate accumulation;
PLA,	placebo;
PPO,	peak power output;
PTV,	peak treadmill velocity;
RER,	respiratory exchange ratio;
RPE,	rate of perceived exertion;
RPM,	revolutions per minute;
RQ,	respiratory quotient;
SS,	steady state;
T1R3,	taster receptor type 1 member 3;
TT,	time trial;
W,	watts.

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Chapter 1

General Introduction

1.1 Background

The benefits of carbohydrate ingestion on exercise performance has been well documented for both prolonged (>2hours) and high intensity (75% $\dot{V}O_{2max}$) bouts of exercise (Coyle *et al.,* 1986; Foskett *et al.,* 2008), as it reduces the rate of liver glycogen depletion and slows the fatty acid utilisation rates (Hargreaves *et al.,* 2004:, Jeukendrup *et al.*, 1999; Jeukendrup *et al.,* 2000; Stellingwreff *et al.*, 2007; Van Loon *et al.*, 1999; Tsintzas *et al.*, 1998). More recent studies have shown that exogenous carbohydrates supplementation in the form of carbohydrate mouth rinse (CMR) may improve exercise performance. Early studies focused primarily on the benefits of CMR on cycling time trial performance (circa 1hr) with work by Carter et al., (2004) showing improvements of 2.9% in relation to cycling time trial performance. It has been hypothesized that this performance improvement can be related to the stimulating oral receptors linked activation of the central nervous system via the CMR (Chambers *et al.*, 2008).

Follow-up studies to that of Carter et al., (2004) have investigated the effects of CMR on other endurance sports (Devenney et al. 2018; Germaine et al. 2018; Dorling et al., 2013; Kasper et al., 2015; Rollo et al., 2008; Rollo et al., 2010); with Rollo et al., (2008) demonstrating positive benefits to running performance with subjects covering more distance in the first 5 minutes of the trial while also having a 1.7% increase in total distance covered. With most of the aforementioned work looking at the benefits of CMR in a fasted state, work into the benefit of CMR in a fed state was carried out initially by Beelen et al (2009). The study showed no significant improvements to cycling TT performance with CMR (6.4% maltodextrin) while in a fed state, citing no difference in the time to completion, heart rate or power output. Improvements of 5.6% were however reported by Devenney et al (2016) for cycling TT performance with the use of CMR (6% solution) while in a fed state.

With studies initially investigating the effects of concentrations of 6% or 6.4% which would be consistent to the %CHO in most available isotonic sports drinks, more recent studies looking at higher concentrations in the range of 10-16% (Lane *et al.,* 2012; Devenney *et al.,* 2016). Improvements of 1.8% were reported in time trial performance with a 10% CHO solution with participants monitored in a fed state, while improvements of 3% were reported in a fasted state (Lane *et al.,* 2012). Devenney et al (2016) investigated the effects of both a 6% and 16% CHO solution had on cycling time trial performance in a fed state. The authors reported improvements of up to 6.1% with a 16% solution in comparison to a placebo. These findings compare favourably to previous studies of 6% and 6.4% solutions, showing improvements in exercise performance linked to oral receptor activation.

The benefits of caffeine ingestion on repeated sprint performance for both running (Carr et al. 2008; Germaine et al. 2018; Mohr et al. 2011) and cycling (Lee et al. 2011) are well documented, with Kalmar (2005) reporting that any performance enhancement may be due to a mechanism of the motor unit activity along with the adenosine receptor antagonism. Recent work by Kasper et al., (2016) investigated the effects of CHO rinsing and caffeine on high intensity interval running capacity in a carbohydrate restricted state, with results showing that CMR and caffeine ingestion increased the exercise capacity of individuals during HIT when compared to a placebo and a standalone CMR. With the initial studies of CMR showing beneficial improvements, the impact of caffeine mouth rinse (CAFF) was then explored. Beaven et al (2013) compared the effects of CMR and CAFF in repeated sprints with recreationally trained males, showing that although no significance was observed in relation to HR and RPE, CMR increased peak power output (PPO) of sprint 1 and both CMR and CAFF trials increased mean power output of sprint 1 when compared to a placebo (ES 0.81-1.08). The results also showed further enhancements in PPO when utilising a combo of CMR+CAFF when compared to just CMR. Similar studies by Kizzi et al (2016) and Lesniak et al (2016) have continued looking at the effects of caffeine rinse on cycling performance, looking at repeated sprints and cycling TT performance respectively. Kizzi et al (2016) reported that CAFF reduces the performance decrement of power output in repeated sprints whilst findings from Lesniak et al (2016) showed no significance in CMR, CAFF or CMR+CAFF in cycling endurance performance in female athletes.

1.2 Aims and Objectives.

The overall aim of this thesis is to determine whether nutritional aids, in the form of carbohydrate mouth rinse, caffeine supplementation and caffeine mouth rinse, can impact exercise performance when athletes are in a fed state. Previous research already established the benefits of CMR and CAFF when athletes are fasted. It is hoped that this body of work can translate from the scientific field to the practical setting for athletes and coaches alike, allowing improvements in training stimulus without the consumption of exogenous supplements, while also providing an alternative to athletes noting GI discomfort/stress.

The aim of this thesis will be achieved by:

Determining whether various concentrations of CMR can elicit a positive effect on cycling time trial performance while athletes are in a fed state (Study 1/Chapter 4).

Determining whether CMR and caffeine supplementation can improve time to fatigue in relation to steady state running and high intensity intervals (Study 2/Chapter 5).

Determining whether CMR and CAFF can improve PPO in repeated cycling sprints while in a fed state (Study 3/Chapter 6).

Chapter 2

Literature Review

2.1 Historical Context

The importance of fuel utilization on various exercise modalities has been well documented over the last century. Early work denoted that a change in respiratory quotient (RQ) from a 0.75 at rest to 0.95 during exercise could signal a shift in carbohydrate metabolism (Chaveau 1894), but also noted that as the RQ was below 1.0 that there was still an element of fat utilization. Work by Christensen and Hansen (1939) demonstrated how varying dietary intake can impact prolonged exercise, with additional carbohydrate supplementations leading to 210 minutes of exercise time compared to 80 minutes with a fat diet. The study also showed by measuring CO_2 and blood lactate that the RQ reported by Chaveau (1896), gives a true representation of fuel utilization. With the use of muscle biopsies, work by Bergstrom and colleagues (1967) determined that increase in carbohydrate intake during moderate exercise can lead to increase in muscle glycogen stores. Work by Costil et al., (1973), was one of the first studies to look at CHO oxidation during exercise, showing that small quantities of the ingested CHO was metabolised and that CHO feedings have little impact on muscle metabolism. These early studies set the foundations to which work by Asker Jeukendrop was able to build on, which one study in 1999 investigating the effects of CHO supplementation had on endogenous glucose production (Jeukendrop *et al*., 1999). CHO feedings in small quantities were able to suppress endogenous glucose production while large quantities fully prevented endogenous glucose production. The studies lay the foundations by which research over the last 20 years could evolve, investigation the benefits of ergogenic aids like carbohydrate and caffeine.

With the nutritional research ever evolving and athletes looking to gain that extra edge in relation to performance, more focus has been put on ergogenic aids and their impacts on performance. These aids would include beetroot juice, beta alanine, whey protein, sodium bicarbonate, carbohydrates, and caffeine, with these aids being administered in various forms (Maughan, 1999). The traditional form of carbohydrate supplementation and caffeine administration has been through the ingestion of tablets/capsules or via the consumption in solution format (Maughan, 1999). More novel forms like gels, gum, sprays and mouth rinse have been researched in the last decade, with research demonstrating the ergogenic benefits in relation to exercise performance (Evans *et al*., 2017; Kizzi *et al.,* 2016). The current literature review will highlight some of the findings for nutritional aids of carbohydrate and caffeine mouth rinse.

2.2 Carbohydrate Pathways

The benefits of CHO supplementation on both prolongs bouts of exercise (>2hrs) and high intensity exercise (>75% $\dot{V}O_{2max}$) have long been documented (Coyle *et al*., 1986:, Foskett *et al*., 2008), with the some positive effects including the reduced rate of liver glycogen depletion and the utilisation rates of free fatty acids (FFA) as a metabolite (Hargreaves *et al*., 2004; Jeukendrup *et al*., 1999; Jeukendrup *et al*., 2000; Stellingwerff *et al*., 2007; Van Loon *et al*., 1999; Tsintzas *et al*., 1998). Although these improvements are well documented, there are however limitations regarding CHO oxidation rates, which include both CHO intake and the absorption rate in the gastro-intestinal tract, and the uptake and utilisation rates of glucose within the muscle itself. An optimal dose of CHO may be required to elicit any positive impact on exercise performance, with this dose set to allow a maximal rate of CHO oxidation, with the dose believed to be 60-70 $g \cdot hr^{-1}$ (Jeukendrop 2008). It is hypothesised that an increase consumption of CHO beyond that optimal rate will not improve oxidation rates but hinder the intestinal absorption due to the over saturation of specific transport proteins. However, the use of a multi-CHO supplement (glucose/fructose combination) can result in higher CHO oxidation rates (up to 43% improvement) due to the multiple transport proteins involved in the uptake of CHO, in this case GLUT 4 and GLUT 5 (Jeukendrop 2008). With increased quantities of CHO ingestion and potential decrease rates of intestinal absorption due to over saturation of transport proteins can lead to cramping while the viscosity of the solution can also be a hindrance as more thicker solutions require fluid from local cells/tissues to dilute the solution hindering the fluid delivery (Jeukendrop 2008), with these issues commonly seen in endurance events like marathon running and stage cycling. With an increase in levels of both plasma glucose and insulin throughout exercise, there is a remarkable ability for the skeletal muscle of trained individuals to absorb and oxidise glucose present, due to the training induced effects of a greater capillary density within the muscle and improved levels of GLUT 4 transporters. However, if levels of absorption are limited in the GI tract, then delivery to the contracting muscle is also hindered (Hargreaves 2004). With this possible risk of GI problems or GI distress among other issues, CHO ingestion can be replaced by CMR during sporting scenarios with CMR being as effective as CHO ingestion in improving glucose levels whilst also improving sports performance.

2.3 Caffeine Pathways

Caffeine is a popular supplement amongst athletes due to its availability in a range of ergogenic aids including sports drinks and energy gels, and it's benefits on exercise performance (Rivers *et al*., 1907). Caffeine is absorbed through the GI tract and metabolised in the liver by P450 1A2 (CYP1A2), with a serum concentration of 0.25-2 mg/L post coffee consumption (Madeira *et al*., 2017). Although the ergogenic effect of caffeine on exercise performance have been well researched over the last two decades and any benefits that caffeine does elicit may be due to the central and peripheral systems, the mechanisms by which caffeine works are still unclear. One hypothesis is that caffeine supplementation can lead to an increase level of activity of the sympathetic nervous system and therefore increased levels of catecholamines (Rachima-Moaz *et al*., 1998), with increased adrenaline levels potentially impacting glycolytic pathways. Another hypothesis is that caffeine supplementation can lead to increased levels of lipolysis and lipid oxidation from increased levels of adrenaline but this pathway during exercise remains in debate (Graham *et al*., 2008). The benefits on physical performance reported, caffeine as a stimulant can also impact mental energy via the CNS, in particular the adenosine receptors. Caffeine doses of 100mg have been shown to increase activity of neural pathways of the brain that are associated to attention and cognitive function (Koppelstaetter *et al.,* 2008). These improvements in mental energy can be related to increased levels of alertness and ability to concentrate (Liebermann 2007).

2.4 Fatigue and Exercise

Fatigue, defined as a physical/mental state of weakness or tiredness and generally co-exist, can be caused by a number of contributing factors mainly depletion in liver and muscle glycogen levels, increased dehydration levels and even the body entering a state of hypoglycaemia. Research has shown that fatigue, mainly peripheral muscle fatigue, is the body's inability to meet the energy demands associated with increased levels of activity. With glucose being the primary fuel source during high intensity exercise, it's lack of availability requires the body to delve into other fuel stores (lipids, amino acids, and ketone bodies) to provide sufficient energy in order for the body to maintain a certain level of function. Without a constant supply of glucose, normal bodily function becomes difficult to maintain, while there is a limited level of activity with neural tissue within the brain, leading to central fatigue. The CNS's inability to activate motor neurons to a desired level means central fatigue hinders performance levels by initiating feeling of tiredness, pain, and lethargy (Davis *et al.*, 1997), with can be more commonly referred in endurance athletes as "hitting the wall". Throughout these events efficiency of primary muscles and joints and core temperature receptors become compromised, leading to increased levels of stress sue to inadequate fuel and hydration levels. Prolonged exercise (3-hour cycling) sans glucose can result in entering a state of hypoglycaemia and decreased levels of force production and maximal muscle contraction (Nybo, 2003), with these decreased levels of force production linked to diminished activity of the CNS. By maintaining blood glucose levels via CHO supplementation, central fatigue can be kept at bay and power output levels can be preserved at the latter stages of prolonged exercise, while maintaining blood glucose levels also leads to a constant supply of glucose to the brain which is required for the uphold of normal brain and body function (Nybo 2003).

2.5 Mechanisms of Carbohydrate Mouth Rinse

The mechanisms by which CMR works are unknown, but it has been hypothesised that reward pathways of the brain are activated by oral receptors sensitivity of CHO caloric content rather than sweetness (Chambers *et al.*, 2009). With the use of fMRI, similar cortical responses were produced by both glucose and maltodextrin as a resultant of the caloric content rather than their perceived sweetness (Chambers *et al.*, 2009). Furthermore, images demonstrated similar activation of regions, primarily in the primary taste cortex and medial orbitofrontal cortex which is defined as the putative secondary taste cortex (Rolls 2007). Images also revealed levels of activation of both the orbitofrontal cortex and anterior cingulate cortex by a few stimuli (Araujo & Rolls, 2004), it is therefore believed that oral stimuli are represented in this region. The possibility that activation was due to viscosity of each solution was voided, as solutions of a similar viscosity (1-2CP of 18% Maltodextrin v control, Chambers *et al.*, (2009)) and different viscosity (2CP 18% Sucrose v 50CP of carboxymethylcellulose, Araujo & Rolls 2004), had similar neural activation levels. Finally, an investigation into the activation pathways regarding different concentrations of sucrose compared to artificial sweetener (sucralose) showed levels of activation in both the FO and AIC, known as the primary gustatory cortex, in both the sucrose and sucralose solutions. Sucrose also elicited a response in other regions, including left ventral striatum, left dorsal candate nucleus, bilateral midbrain, and right thalamus, all of which are contributors to the taste rewards pathway. It was therefore hypothesised that sucrose may not act solely on T1R3 taste receptors (Damak *et al.*, 2003), and that reasoning for a stronger response to sucrose in relation to brain activation could be influenced by the brain sensing blood glucose levels (Levin 2006).

2.6 Carbohydrate Mouth Rinse and Performance

CMR has been demonstrated to be most effective during the latter stages of a 1 hr cycling TT as there are changes in energy output from levels of high to low, with this statement backed by an increasing amount of evidence (Carter *et al.,* 2004; Chambers *et al.,* 2009; Pottier *et al.,* 2010). The first study to investigate the effects of CMR is that by Carter and colleagues (2004), which looked at the effects of a 6.4% maltodextrin bolus on cycling TT performance (time trial was a %work max test, calculated by an equation devised by Jeukendrup et al., (1996), with CMR given to subjects every 12.5% of the trial completed. Performance times between the CMR and PLA trials were 59.57 ± 1.5 min & 61.37 ± 1.56 min respectively which denoted an average improvement of 2.9% in the CMR trial, potentially due to the significant improvement in power output between each trial (259 ± 16W CMR V 252 ± 16W PLA). Further studies by Pottier et al., 2010, using similar cycling and CMR protocols demonstrated an improvement of 3.7% in cycling performance, although this study did use alternative CHO supplements in the form of monosaccharides and disaccharides (Pottier *et al*., 2010). Recent work by Jensen and colleagues (2018) looked at the effects of CMR on steady state cycling and TT performance. Participants received a pretrial snack and CHO feeding (30g per hour) throughout the SS cycling before undergoing a TT. Results showed no significant difference in the CMR compared to the PLA, but the study did report a 1.7% improvement in TT performance (Cohen's d = 0.21), which equates to a 35s improvement. Another important study is that by Chambers and colleagues (2009), who again investigated the effects of glucose and maltodextrin rinse protocols on exercise performance but also with the use of fMRI to try identifying which brain regions are stimulated by the solutions. The study showed that there was quicker time to completion of the cycling protocol for the glucose trial compared to the PLA (60.4 ± 3.7 min & 61.6 ± 3.8 min respectively) and for the maltodextrin trial compared to the PLA (62.6 ± 4.7 min & 64.6 ± 4.9 min respectively), with increased levels of power output in both CHO trials compared to the PLA. MRI images revealed that oral exposure to both glucose and maltodextrin activated several areas of the brain, some of which are believed to be involved in the reward pathways and the regulation of motor activity, with results supporting the hypothesis of the presence oral receptors that are sensitive to carbohydrate and independent of sweetness. Murry and colleagues (2018) investigated the effects of CMR on cycling TT and plasma insulin levels. Using a 6.4% CHO solution, the study showed improvements in 40km TT performance which agrees with previous work (Carter *et al.*, 2004; Pottier *et al.*, 2010; Phillips et al., 2014). The study showed no difference in plasma insulin levels when comparing the CMR to PLA trials, with plasma insulin monitored across the first 25km of the 40km trial (Murray *et al.,* 2018). There are however studies that have reported contradicting findings, with two studies showing no improvement in TT performance with CMR when compared to a PLA (Beelen *et al*., 2009; Whitham and McKinney, 2007). Other work by Pires and colleagues (2018) reported no improvements in 4km TT performance when comparing CMR to PLA. The study reported similar power outputs and time to completion for the distance across the two trials. Although no performance improvements were reported, the study did report a large effect size (Cohens $d = 1.0$) when looking at twitch interpolation, which could suggest CMR may hamper a central fatigue development in comparison to the PLA (Pires *et al.*, 2018).

Work by Chong and colleagues (2010), moved away from the traditional cycling TT protocols outlined in the above studies, instead investigating the effects of CMR had on sprint performance in cycling. Again, comparing CMR in the form of a glucose trial and maltodextrin trial to a PLA trial, the observed no significant difference in terms of power output across the trials. More recent work by Beaven and colleagues (2013) used similar methodology to that of the study by Chong, showed that CMR can improved PPO in sprint 1 of the trial compared to the PLA, meaning there could be a benefit for short specific sprint performance but also deduced that CMR could have greater benefits if there is a substantial recovery between sprints.

2.7 Carbohydrate Mouth Rinse & Running Performance

The first initial study to investigate the effects of CMR on running performance was that by Whitham and McKinney (2007), which compared a 200mL bolus of 6% CHO to a PLA with the study showing no significant difference in O2 kinetics, RPE and HR values across the two trials. Rollo et al., (2008) investigated the effects of CMR on a 30-minute run protocol, comparing a 6% CMR to a taste matched PLA. Results showed a greater distance covered in the CMR trial along with a higher self-selected speed in the first 5 minute of the trial compared to the PLA. Further work by Rollo et al., (2010) investigated the effects of a 6.4% CHO and electrolyte solution on 1-hour running performance, with subjects rinsing a bolus at set timed intervals of the running protocol (15min, 30min, 45min). Although no differences were reported in RER values or in blood glucose concentrations, the CHO+E trial did cover a greater total distance when compared to the PLA trial. More recent work by Kasper et al., (2016) investigated the effects of CMR and CAFF supplementation on steady state running and high intensity interval running in a fasted state, with the protocol constriction of a 45-minute SS run and intervals of 1min @80% $\dot{V}O_2$ max interspersed with 1 min of walking at 6 $km.hr^{-1}$. Significantly greater time to fatigue was witnessed in the CMR trial (52 ± 23 min) compared to PLA (36 ± 22 min).

2.8 Fed v Fasted

The benefits of CMR have been well documented across the literature, showing improvements in endurance exercise (cycling TT performance) and high intensity exercise (repeated sprints), with most studies looking at the benefits of CMR in a fasted state. However, recent work investigating the benefits of CMR in a fed state due to the lack of exercise performance being carried out in a fasted state in a practical setting because of a lack of substrate availability. One such study that investigated the effects of CMR in both a fed and fasted state is that by Fares and Kayser (2011), looking at the effects on time to fatigue in cycling. Although no significant difference was reported in physiological metrics, the use of CMR increased time to fatigue in a fasted state by 7% and fed state by 3%. Further work by Lane et al., (2012), who again compared the effects of CMR on a 60-minute cycling TT, comparing the use of a 10% CHO solution in a fed and fasted state. Results showed that CMR increased mean PO by 1.8% in the fed trial and 3.4% in the fasted trial compared to the PLA, concluding that although CMR in a fasted state has a greater effect, it's use in a fed state can still contribute to optimal performance (Lane et al., 2012). However, work by Beelen et al., (2009), showed no significant difference in time to completion when comparing a 6% CMR to a PLA trial, with participants in a fed state.

2.9 Caffeine Supplementation

Caffeine supplementation can enhance both repeated sprint performance in running (Carr *et al*., 2008; Mohr *et al*., 2011) and cycling (Lee *et al*., 2011) along with effects on perceptions of physical work in various formats of exercise performance (Dolan et al., 2017), with this endurance and sprint performance enhancement potentially due to a mechanism of the motor unit activity along with the adenosine receptor antagonism (Kalmar 2005). The addition of a caffeine solution to CMR was first considered by Beaven and colleagues (2013), by incorporating a 1.2% CAFF to the 6% CMR and determining it effects on cycling sprint performance. Results showed that the combination of solutions significantly improved athlete's PPO in Sprint 1 and MPO in Sprint 6 of the trial. Additionally work by Kizzi and colleagues (2016), investigated if a 2% CAFF solution could enhance cycling sprint performance when compared to a placebo. CAFF did maintain exercise performance and prevent the fatigue drop-off when compared to the PLA, however no significant difference was observed in power output when compared to the CONT. Work by Lesniak (2016) compared a 6% CMR, 1.2% CAFF, and a CMR+CAFF combination to determine the effects on cycling TT performance (60-minute protocol). No significant differences were observed in power outputs or time to completion across all three trials, although one caveat in this study was the lack of a PLA trial, therefore it is unknown if these trials can enhance exercise performance. CAFF compared to a PLA appears to improve repeated sprint performance (1.4 % decrease v placebo), with increased HR levels but no significant difference recorded for RPE (Glaister *et al*., 2008). Evans et al., (2017) explored the effects of caffeinated gum on repeated sprint performance, consisting of 10 x 40m sprints with a 30s recovery. Results showed that the caffeine trial improved sprint performance decrement compared to a PLA (5.53±3.12% vs. 6.53±2.91%) in low habitual caffeine users, although no differences were reported in moderate or high habitual users. More work by Kasper and colleagues (2016), looked at time to exhaustion following 45 min steady state running was measured, with findings showing a that the CMR+CAFF trial had significantly greater time to fatigue (65 ± 26 min) when compared to the PLA trial (36 ± 22 min). Recent work by Pereira and colleagues (2021) investigated the effects of CAFF, CMR or CAFF+CMR on resistance-based exercises. Using physically active females, the study showed that all interventions improved performance of resistance training compared to PLA, with CAFF also showing a decreased in RPE compared to the other interventions (Pereira *et al.*, 2021). Van Cutsem and colleagues (2018) investigated the effects of CAFF and MALT had on cognitive function and mental fatigue. Using a 90minute Stroop task, the authors reported that CAFF & MALT induced mental fatigue to a lesser extent than that of the PLA. The potential mechanisms were accounted for the ability of CAFF & MALT to counter mental fatigue, were the absorption of either supplement in the oral cavity which could lead to signalling responses of brain pathways. This information would agree with the previous findings of Chambers and colleagues (2009).

2.10 Summary

Literature has shown that CHO supplementation, whether ingestion or CMR, can elicit a positive effect on athletic performance during prolonged bouts of high intensity exercise and high intensity intervals. CMR has been shown to have positive effects on performance in different sporting formats, with most research indicating oral rinsing with 6% CHO solution can enhance cycling TT performance by 2-7%, with the study by Beelen (2009) one exception. Similar findings have been shown with CMR and running performance, with improvements of 1.7% in distance covered with the use of CMR (Rollo *et al.,* 2008). The addition of caffeine to the CMR protocols has also been shown to improve exercise performance, in terms of time to fatigue and power output, without impacting physiological responses like RPE, blood glucose or elevated HR levels. Research does support the hypothesis that oral receptors sensitive to CHO can initiate activation of neural pathways, including those involved in the reward pathways and the primary and secondary taste cortex. Improvements in performance related to these oral receptors and neural activation have been achieved whilst individuals are in a fasted state (Chambers et al., 2009), however further research is required to determine if the same levels of activation can be achieved throughout exercise when individuals are is a fed state.

Chapter 3

General Methodology

3.1 GENERAL METHODOLOGY

3.1.1 Location of testing and ethnical approval

All of the exercise testing was carried out in the physiology laboratory in Technological University Dublin Tallaght. The ethical committee of Technological University Dublin Tallaght approved all experimental procedures and exercise protocols.

3.1.2 Subjects

All subjects who participated in each trial were young healthy and recreationally active males. An overview of participants physical and physiological characteristics can be viewed in Table 3.1. All subjects were given full details of the demands of the protocols and gave written consent. Participants refrained from any additional intense exercise outside of the exercise requirements involved in the study and refrained from the consumption of alcohol and caffeine in the 24 hours prior to each exercise testing session. All participants had no history of neurological or cardiovascular disease and also reported no musculoskeletal injuries in the previous 6 months of the study.

Table 3.1 Subjects physical and physiological characteristics

	Study 1	Study 2	Study 3
Age (years)	22 ± 7	23 ± 3	22 ± 3
Height (m)	1.75 ± 0.07	1.75 ± 0.05	1.75 ± 0.07
Weight (kg)	69 ± 9	78 ± 7	75 ± 10
$\dot{V}O_2$ max (ml·kg^{-1}·min^{-1})	51 ± 3	51 ± 3	53 ± 6

3.1.3 Anthropometry

Each participants height and body mass was measured on their first visit to the laboratory which coincided with their physiology assessment. Subjects height (SECA 217, Birmingham, UK) and weight (SECA 762, Birmingham UK) was assessed on each participants initial visits, with both height and weight measured on each visit thereafter and any major changes were noted.

3.2 Cardio-Respiratory Measurements

3.2.1 Heart Rate

Subjects were fitted with a short-range telemetry system for the measurement of heart values (Polar T31, Polar Electro, Kempele, Finland) across all exercise trials.

3.2.2 Assessment of respiratory gases during exercise

Subjects were required to wear a facemask for measurement of respiratory gases during initial physiology assessment. Expired oxygen and carbon dioxide were analysed during breath-to-breath analysis via a Cosmed Quark CPET gas analysis system (Cosmed, Rome, Italy).

3.3 Assessment of Maximal Oxygen Uptake ($\dot{V}O_2$ max)

3.3.1 Bike Protocol

All subjects were assessed for $\dot{V}O_2$ max using an incremental exercise test performed on a Wattbike Pro (British Cycling Wattbike, Nottingham, England). Oxygen uptake ($\dot{V}O_2$ max) was measured during exercise by breath-by-breath analysis using a Cosmed Quark CPET system (Cosmed, Rome, Italy) after being calibrated with known reference gases. The incremental test consisting of a 5 min warm up at 100 W, followed by increments of 25W every 2.5 minutes until either volatile exhaustion or the power output demands could not be met for a 15 second period. The $\dot{V}O_2$ max was taken as the highest value obtained in any 30 second period and was stated as being achieved by the following end-point criteria: 1) heart rate within 10 beats of age-predicted maximum, 2) respiratory exchange ratio > 1.1, and 3) plateau of O2 kinetics despite the increased workload.

3.3.2 Treadmill Protocol

All subjects were assessed for $\dot{V}O_2$ max using an incremental exercise test performed on a T170 treadmill (Cosmed, Rome, Italy). Oxygen uptake ($\dot{V}O_2$) was measured during exercise by breath-by-breath analysis using a Cosmed Quark CPET system (Cosmed, Rome, Italy) after being calibrated with known reference gases. The The protocol used was based off that of Akubat et al, (2014), consisting of 4-minute stages interspersed with a minute recovery, starting at 8 $km\cdot hr^{-1}$ with increments of 2 $km\cdot hr^{-1}$ after completion of each stage (8, 10, 12, 14, 16 km·h-1) until onset of blood lactate accumulation (OBLA) which was followed by a RAMP protocol (increments of 1 $km\cdot h\ r^{-1}\cdot min^{-1}$) to volatile exhaustion. Like the bike protocol, $\dot{V}O_2$ max was taken as the highest value obtained in any 30 second period and was determined to be achieved by 1) a plateau of oxygen consumption despite an increase of workload, 2) a RER value greater than 1.1, and 3) heart rate within 10 beats of age-predicted maximum.

3.4 Blood Sampling

Blood lactate levels were monitored throughout each study. Subject as their resting levels recorded prior to each testing protocol, while also having levels measured throughout each exercise protocol and after cessation of the protocol. Blood lactate levels were measured using a Lactate plus meter (Nova Biomedical, Massachusetts, USA).

3.5 Measurement of Psycho-Physiological Variables

3.5.1 Rating of Perceived Exertion

Subjects rating of perceived exertions were recorded throughout the duration of each exercise trial, using a 15 point Borg Scale (Borg 1970). Table 3.2 displays the scale used throughout the trials.

Table 3.2 Borg scale used for subjects rating of perceived exertion (Borg 1970)

Rating	Description
6	No exertion at all
7	Extremely Light
8	
9	Very Light
10	
11	
12	
13	Somewhat Hard
14	
15	Hard
16	
17	Very Hard
18	
19	Extremely Hard
20	Maximal Exertion

3.6 Nutritional & Activity Analysis

Participants were asked to keep a two-day training diary prior to each test visit, being allowed to undertake low intensity exercise (heart rate below 150 beats/min) for up to 2 hours. Along with the training diary, a two-day dietary diary was recorded to ensure participants were fed, while also acting as a tool to ensure participants consumed the same diet on visits 2, 3, and 4. Participants were also asked to refrain from consuming alcohol & caffeine in the 24hrs prior to each testing session. Participants consumed a meal 2-3 hours before testing (49 ± 2% carbohydrates, 18 ± 1 % protein and 33 ± 2% fat) with each meal recommended to the participants by the authors. Although the dietary diaries were used to replicate diet and recommendations were made for meals on the morning of each test session, the authors can't guarantee these guidelines were adhered to by participants. While participants were asked to refrain from caffeine in the 24hrs leading into each test session, habitual caffeine consumption was not monitored throughout the study. Similarly GI and sleep disturbances were not monitored throughout the studies. As athletes can compete while having GI distress or sleep irregularities, the authors decided not to monitor these factors in an effort to make the research as practical applicable as possible.

3.7 Rinse Detection

Participants were asked at the end of each test session which nutritional intervention they received and for any reasoning behind their decision, with the responses recorded. Participants were informed of the individual trial sequence at the completion of the entire study.

3.8 Wash-out Periods

A 5–7-day wash-out period was implemented between each testing session. The rationale behind this was that test protocols for study 1 and 2 were similar to race scenario or training scenario, and it is unlikely athlete would do these exercises back-to-back. Although the test protocol for Study 3 is reasonable light in volume compared to the protocols in the previous two studies, the authors again decided for the same wash-out period, to mimic training demands placed upon athletes. It was recognized that both CAFF and CHO benefits would have worn off within the 12-24hrs post exercise test, but to make the studies as practically applicable as possible this wash-out period was used, so findings are relatable to coaches/practitioners.

3.9 Statistical Analysis

All statistical analysis was performed using the statistical package for social sciences (SPSS; version 24, IBM, USA). All data in text, figures and tables are presented as means ± SD, with P values < 0.05 indicating statistical significance. Effect size were also used to determine any small worthwhile change, with Cohen's D used to indicate any effect size.

Chapter 4

Effects of various concentrations of carbohydrate mouth rinse on cycling performance in a fed state

4.1 Introduction

The ingestion of carbohydrates and their beneficial effects on exercise performance in both prolonged bouts of exercise (>2hours) and also in high intensity exercise (75% max) has been previously documented (Coyle *et al.,* 1986; Foskett *et al.,* 2008). Carbohydrate ingestion can have a positive impact during exercise as it plays an important role in reducing the rate of liver glycogen depletion and slows the rate by which fatty acids are utilised as a fuel source (Hargreaves *et al.,* 2004; Jeukendrup *et al.,* 1999; Jeukendrup *et al.,* 2000:, Stellingwreff *et al.,* 2007; Van Loon *et al.,* 1999; Tsintzas *et al.,* 1998).

It has been reported that mouth rinsing with a CHO solution works best during the latter stages of a 1 hour cycling time trial, as there is a reduction in power (Carter *et al.,* 2004.). Carter et al., (2004) investigated the effects of rinsing a participant’s mouth with a carbohydrate solution (6.4% maltodextrin) compared to a 0% placebo during a 1-hour time trial. Each solution was rinsed around the mouth every 12.5% of trial completed and then expelled to prevent swallowing the solution. Results reported that the use of 6.4% CHO solution meant a 2.9% improvement in average time to completion when compared to the placebo results. Carter et al., (2004) hypothesised that mouth rinsing with a CHO solution can improve time trial performance through the activation of oral receptors that can trigger the reward pathways of the brain and body, hence the improvement in the latter stages of the TT.

The majority of studies that investigate the effects of CHO mouth rinsing on performance have been focused on cycling performance, there have been studies which have investigated the effects of CHO mouth rinsing on other endurance sports (Dorling *et al.,* 2013; Kasper *et al.,* 2016; Rollo *et al.,* 2008; Rollo *et al.,* 2010). Rollo et al., (2008) investigated the effects of CHO rinsing of running performance after an overnight fast, using similar administering and expelling techniques used in previous studies, giving a 25ml bolus at predetermined stages in both the warm-up and time trial. The overall study results showed that CHO rinsing has a positive effect on running performance, with running speeds quicker in the first 5 minutes of the 30-

minute trial in the CHO group when compared with the placebo group. It was also indicated that the CHO group covered more distance in the first 5 minutes of the trial while also having a 1.7% increase in total distance covered when compared with the placebo group. With the study utilising a self-selected pace based on RPE (value of 15), increased speeds in the first 5minutes could again be related to increased activation/excitement of neurological pathways (Carter et al., 2004; Chambers et al., 2009), but the true mechanism behind the increased speeds are unknown. Recent work by Kasper et al., (2016) investigated the effects of CHO rinsing and caffeine on high intensity interval running capacity in a carbohydrate restricted state. After completing a glycogen depletion test 24 hours prior to the exercise session, participants completed a 45min steady state run (65% $\dot{V}O_{2max}$) followed by a HIT exercise capacity test consisting of 1 minute at 80% followed by a minute at 60% until the onset of fatigue. The study showed that the use of CHO rinse and caffeine ingestion increased the exercise capacity of individuals during HIT when compared to a placebo and a standalone CHO rinse.

Not all the studies which have investigated CHO rinsing have discovered positive findings. Beelen et al., (2009) showed no enhancement in performance when mouth rinsing a CHO solution (6.4% maltodextrin) while in a fed state, while there was also no difference when comparing the CHO solutions to the placebo solution. The results showed no significant difference in performance times in the CHO or PLA group, while also stating no difference was seen in the heart rate or power output.

The majority of CHO rinse studies have investigated concentrations of 6% or 6.4%, with more recent studies looking at a higher concentration (Lane et al., 2012). A 1.8% improvement in time trial performance were noted when a 10% CHO solution was rinsed for 10 seconds with participants monitored in a fed state, with 3% improvements shown in a fasted state (Lane et al., 2012). These findings compare favourably to the previous research findings of the 6% and 6.4% studies, showing an improvement in performance linked to activation of oral receptors.

It has been well documented to date that mouth rinsing of a 6% or 6.4% CHO solution can have a positive effect in relation to performance in a fasted state it is however unclear the effect of this concentration in a fed state. The aim of the current study is to develop the foundations set by Beelen et al., (2009) and Lane et al., (2012) by investigating the effects of rinsing with the traditional CHO rinse solution (6%) and a higher concentration (in this situation a 16% carbohydrate solution) have on exercise performance when compared to a 0% solution while participants are in a fed state. By using a 6% and 16% solution, the current study is able to compare two solutions which can replicate two commercially available sports drinks and determine their benefits and also to determine if there is a concentration dependence with regards to exercise performance and one could hypothesize that by comparing one concentration to another, performance improvement could be further enhanced with the use of a higher concentration of CHO due to a greater saturation of oral receptors.

4.2 Methods

Recruitment: Twelve recreationally active males volunteered to participate in this study (age 22 ± 7 years, body weight 69 ± 9 kg, height 1.75 ± 0.07 m, body-mass index 22 ± 1.7 kg/m^2, Wmax 260 ± 28 W, $\dot{V}O_{2max}$, 51 ± 3 ml.kg−1.min−1), all of whom had engaged in cycling activities (3-5 days a week/5-8 hours per week). All participants were informed verbally and in written form of the study design and the physiological demands they would be placed under. Each individual completed a medical questionnaire and consent form prior to testing. The study was approved by local research ethics committee.

Overall Study Design: The protocol consisted of four visits to the laboratory with all tests carried out on a cycle ergometer (Wattbike Pro, British Cycling Wattbike, Nottingham, England). Visit 1 involved a RAMP test to determine each participant's maximum aerobic power or work capacity (Wmax). In the remaining three visits, individuals completed a set amount, which was individualised and calculated based on their maximum work capacity, in the shortest timeframe possible. In a randomised, repeated measures and double blinded study participants rinsed a 6% or 16% CHO solution or 0% PLA around the mouth at predetermined intervals.

Activity and Diet before Experiments: Participants were asked to keep a two-day training diary prior to each test visit, while they were allowed to undertake low intensity exercise (heart rate below 150 beats/min) for up to 2 hours. Along with the training diary, a two-day dietary diary was recorded in order to determine if participants were fuelled coming into the test sessions. Participants were also asked to refrain from consuming alcohol in the 24 hours prior to each visit and refrain from caffeine the day of each visit. The dietary diaries were a tool used to ensure participants consumed the same diet on the 2^{nd}, 3^{rd} and 4^{th} visit measure as a measure to ensure repeatability. Participants consumed a mixed meal (eg. Meals recommended were a chicken and pasta with tomato sauce or a spaghetti Bolognese) 2-3 hours before testing (49 ± 2% carbohydrates, 18 ± 1 % protein, and 33 ± 2% fat) with each meal recommended to the participants by the authors. Although two-day dietary diaries were recorded and meals were recommended to each participant, the authors cannot guarantee participants adhered to these guidelines.

Maximum Workload Capacity Protocol: The maximum aerobic capacity test is a modified protocol which was based on the protocol performed by Beelen et al., (2009). Each participant performed an all-out incremental exercise test which was used to determine their Wmax. Each participant underwent a 5-minute warm-up at 100W. On completion of the warm-up, the workload was set at 150W and increased by 25W every 2.5 minutes until the onset of exhaustion instead of the 50W increase (Pottier *et al*., 2010). Exhaustion was determined by either of two factors, 1) drop off in cadence by more than 15 RPM for 15s period or 2) inability to maintain target wattage for a continuous 30s period. The increased workload of each stage throughout this protocol was influenced by an increase in cadence. Heart-rate (Polar RS200, Polar Electro, Finland), RPE and cadence were recorded on the completion of each interval. Wmax of each individual was calculated using the following formula; *W out + (t/150) x 25,* where W out is the watts of the last complete stage and t is the time spent in the final unfinished stage (Kuipers *et al.,* 1987).

Time-Trial Protocol: Prior to the commencement of the test protocol, each participant was weighed, and baseline blood lactate levels were recorded and individuals were fitted with a heart-rate monitor (Polar RS200, Polar Electro, Finland) which was linked with the Watt bike. Participants endured a 5-minute warm-up at approximately 40% of Wmax, during which they were familiarised with the BORG scale of perceived exertion (RPE) (Borg 1982; Borg 1990). Following completion of the warm-up, participants were asked to complete a set amount of work in the quickest time possible. The total amount of work to be completed was calculated using a modified version of Jeukendrup et al., (1996) equation.

Total amount of work in Joules = 0.65 X Wmax X 3,600.

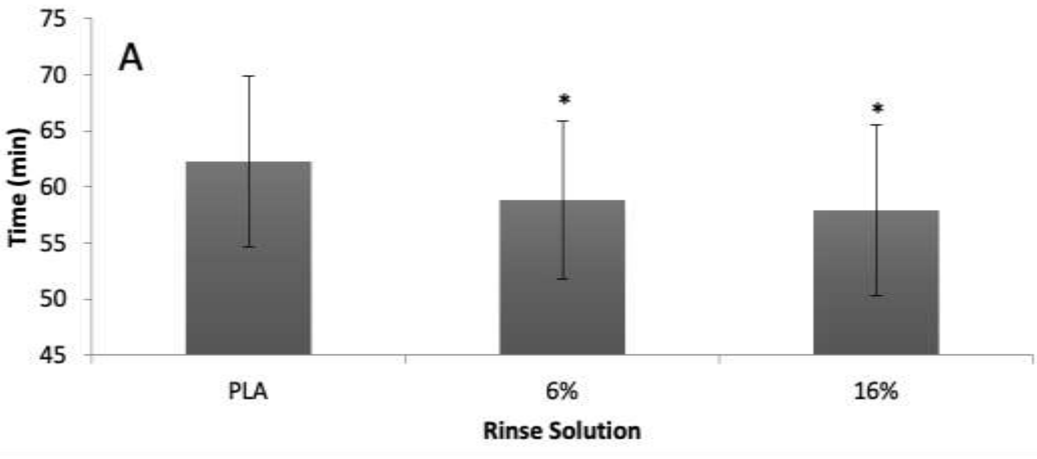
The equation by Jeukendrup et al., (1996) calculated 75% of the participants Wmax for endurance trained athletes, although due to recreational nature of the participants in the study and with evidence from previously unpublished work, the authors sought to modify the equation to 65%. The time trial protocol employed is designed to standardise workload so that each subject takes approximately 1 hour to complete the work. The wattbike was kept at a uniform resistance (resistance of 4) throughout the time trial performance for each participant to maintain a similar intensity for each participant. Participants were only able to view the total amount of work they had performed, with heart rate, time and cadence values blocked from view and clocks removed to prevent participants knowing the time. No encouragement was offered throughout the test and the only interaction was when solutions were given for rinsing at 12.5 % completed intervals, or to record HR, cadence and RPE at every 12.5% of completion. Laboratory conditions were held constant (ambient temperature 18-21°C) throughout each trial, with participants cooled using an electric fan.

Mouth Rinse Protocol: Over the three time-trial visits, participants would use a 0% (placebo), 6% or 16% CHO solution (maltodextrin, due to lack of colour and taste). Each sample was a 25ml bolus which was weighed before and after mouth rinsing, which was to ensure none of the sample was ingested. A bolus was provided to the participant after the warm-up and every 12.5% of completion in the trial. The solution was rinsed around the mouth for 5 seconds before being expelled into a pre-weighed container. 6% and 16% solutions were used in the study as they equated to the CHO content in a traditional sports isotonic drink (Lucozade Sport) and a traditional energy drink (Lucozade energy), which are readily available to coaches/athletes. Solutions were made by an external researcher who was not affiliated to the study, to ensure the trial remained a double-blind study.

Statistical Analysis: All collected data was analysed using SPSS (Version 22.0, Chicago, IL). The variables were compared using a one-way repeated measure ANOVA, which was done to examine the effects across the three time-trial performances and their corresponding solutions (0% placebo, CHO 6% & CHO 16%). Overall time of completion, average power output, cadence and speed were compared between each trial. Heart rate and RPE were compared at each individual stage across all three tests and were analysed using two-way repeated measures ANOVA. All data is represented using mean ± standard deviation with significance set at $p < 0.05$.

4.3 Results

Performance time and power output: In relation to time trial performance both CHO solutions were significantly faster in comparison to the PLA trial, as performance times for the 6% CHO versus the PLA trial were 58.8 ± 7.0 min v 62.3 ± 7.6min (p = 0.002) while performance times for the 16% CHO trial versus the PLA trial were 57.9 ± 7.6 (p = 0.001). The individual differences in time to completion across all trials are shown in Figure 1C.



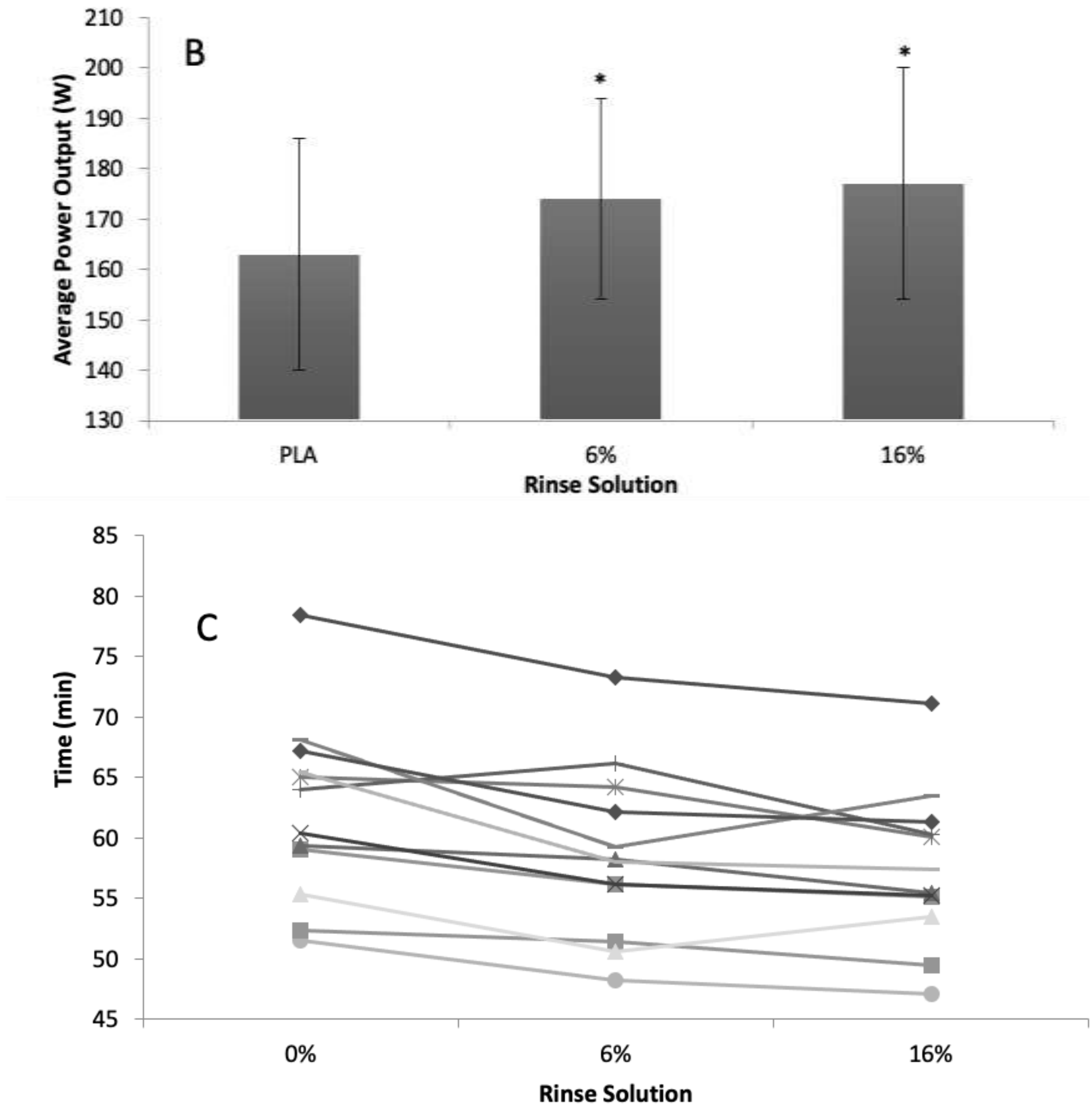


Figure 4.1- Performance times (A), average power output (B) and individual performance times (C) in the placebo and carbohydrate trials with values expressed as Mean ±SD. (A)* statistical difference $p<0.05$; (B)*statistical $p<0.05$.

A significant difference was also observed in average power output and average speed across the three trials. When comparing the 6% trial versus PLA and 16% trial versus PLA, power outputs were 174 ± 20 W v 163 ± 23 W (p = 0.002) & 177 ± 23 W (p = 0.001) respectively. However similar to performance time, no significance was reported in power output when comparing the 6% trial to the 16% trial, (p = 0.291).

No significant difference was observed in the average speed maintained when comparing the 6% and 16% trial ($p = 0.273$), significance was seen when either CHO trial, 6% or 16%, were compared to the PLA trial (34.8 ± 1.6 $km.h^{-1}$ v 34.1 ± 1.7 $km.h^{-1}$, $p = 0.002$, & 35.1 ± 1.8 $km.h^{-1}$, $p = 0.001$). Power output and speed were typically observed to be higher in the first 10 minutes and final 15 minutes across in both carbohydrate trials in comparison to the placebo trial.

Heart rate and RPE: Values for heart rate and rate of perceived exertion (RPE) were seen to increase steadily with the onset of exercise across the three trials. Average heart rate values of the PLA, 6% and 16% trials were 148 ± 18 bpm, 153 ± 20 bpm, and 153 ± 15 bpm respectively with maximal values for the PLA, 6% and 16% trials reaching 168 ± 18 bpm, 171 ± 19 bpm and 174 ± 14 bpm respectively. There were no differences in heart rate responses across the three trials ($p > 0.005$). Like heart rate, RPE values increased steadily throughout the three trials, with average values of 14.3 ± 1.07, 14.2 ± 1.7 & 13.8 ± 1.5, for the PLA, 6% and 16% trial respectively. Maximum RPE values were recorded at 16.3 ± 1.5, 17.4 ± 1.7 & 17.3 ± 1.9, for the PLA, 6% and 16% trial respectively. No significant difference was reported in RPE values across the three trials ($p > 0.005$).

Rinse solution detection: From the 12 participants in the study, 4 were able to distinguish a difference in the mouth rinsing solutions used across the three time-trials, reporting a difference in feel or viscosity of the solutions. Out of the four that reported a difference, three performed better in the 16% trial when compared to both the 6% and PLA trial, while the fourth performed better in the 6% trial when compared to both the 16% and PLA trial. The other 9 participants could not distinguish any difference across the three solutions.

4.4 Discussion

The aim of the current study was to investigate the effects of mouth rinsing with a 6% and 16% carbohydrate solution have on exercise performance in comparison to a 0% solution while participants are in a fed state. The two concentrations used were as they equate to the %CHO in commercial sports drinks, while it was hypothesized that the greater concentration may potentially lead to a great saturation of oral receptors therefore leading to potentially great improved performances. The current study shows that when compared to a placebo, the use of carbohydrate mouth rinse can lead to improvements in performance times, average power outputs and average speed during a time trial performed in a fed state. Early studies have investigated the effects of carbohydrate mouth rinsing on performance during high intensity exercise. Carter et al., (2004) reported improvements of 2.9% in cycling time trial performance with use of a maltodextrin mouth rinse, while improvements of 3.7% were reported when a mono and disaccharide sports rinse was substituted instead of the maltodextrin mouth rinse (Pottier *et al*., 2010). However, studies by Whitham and McKinney (2007) and Beelen et al., (2009) did not support these findings, reporting no difference in performance times when comparing a carbohydrate mouth rinse to a placebo. The studies by Carter et al., (2004), Pottier et al., (2010) and Whitham and McKinney (2007) all investigated the performance benefits of carbohydrate mouth rinsing within a fasted state, while the study by Beelen et al., (2009) looked at the benefits in a fed state. The current study took the same premise as the study by Beelen et al., (2009), with 12 participants performing three separate time trials while in a fed state, where they were provided with a 6% CHO, 16% CHO or PLA mouth rinse solution at every 12.5% of the trial completed. The use of nutritional diaries and same day/time testing enabled the monitoring of each participants fed status. The results show improvements in time to completion in both CHO trials in comparison to the PLA trial, with significant improvements in time of completion in both CHO time trials. This improvement in time of completion is associated with each participant's ability to sustain both greater power output and higher speed during the 6% and 16% trial. Both heart rate responses and session RPE were similar across the three trials. By testing the participants of the current study in a fed state as opposed to a fasted state, the practical relevance of carbohydrate mouth rinsing can be determined.

It is believed that the CHO mouth rinse stimulates the reward regions of the brain via oral receptors due to the caloric content of the carbohydrate solution (Chambers *et al*., 2009; Turner *et al.,* 2014). One would think that this stimulation of the reward pathways would lead to lower RPE levels observed in the CHO trials compared to the PLA. However similar to previous reports no differences were observed in RPE between either CHO trial and the PLA trial. The concentration and rinse duration may also have an impact on the effectiveness of the CHO rinse. The vast majority of the current research has use of a 6% or 6.4% CHO rinse solution for 5 second duration (Beaven *et al.,* 2013:, Carter *et al*., 2004:, Dorling *et al.,* 2011:, Pottier *et al.,* 2010:, Rollo *et al.,* 2008, 2010), while improvements in TT performance have been noted with a greater solution concentration and longer rinse duration in both fed and fasted states (Lane *et al.,* 2012). A greater concentrated solution consisting of 16% maltodextrin was compared to the traditional 6% solution and placebo in the current study. As previously stated, both CHO rinse solutions improved cycling performance in comparison to a PLA (improvements of 5.6% and 7.1% observed), although no significant difference was observed when comparing both CHO trials to each other. Using a higher concentration of mouth rinse may enhance the saturation of the oral receptors leading to a greater stimulation of the reward pathways reported by Chambers et al. (2009) and Turner (2014), although further investigation on the mechanism is required.

4.5 Conclusion

The applications of carbohydrate rinsing have grown since the first study by Carter et al., (2004), with current literature investigating the effects in sports ranging from cycling (Beelen *et al.,* 2009:, Fares *et al.,* 2011:, Lane *et al.,* 2012:, Pottier *et al*., 2010) to running (Rollo *et al.,* 2008, 2010, 2011:, Whitham *et al.,* 2007), with more recent studies investigating the effects on field sport simulation and strength work (Dorling *et al*., 2011:, Jensen *et al.,* 2014:, Painelli *et al.,* 2011). With the ergogenic benefits well documented, the data can translate from the scientific field to the practical setting for athletes and coaches alike, as the current study along with other findings suggest that CHO rinsing at regular intervals may benefit those athletes reporting gastrointestinal problems. In conclusion, the present study shows that the use carbohydrate mouth rinsing during high-intensity exercise can lead to an improvement in exercise capacity during a simulated time trial when exercise is carried out in a fed state.

Key Findings
Results indicate that CMR (6% or 16%) significantly improve time of completion for cycling TT performance (5-7% improvement) when compared to a PLA. This information adds to the current body of work and with subjects in a fed state, results are more practically applicable to coaches & athletes.

Link to Next Experimental Chapter
With the previous research showing the benefits of CMR on cycling in a practical environment (i.e., in a fed state), the authors wanted to build on the foundations laid by the study by Kasper et al., (2016) and determine the effects of CMR and CAFF on running performance. With CHO and CAFF two heavily reliant supplements during endurance running and research showing a potential benefit on SS running and HIT in a fasted state, the study again applies a more practically applicable setting with participants in a fed state. Participant recruitment switched from recreational athletes with a cycling background to recreational runners, as they would meet the demands of the exercise protocol. A 6% CHO solution and 200mg CAFF was used in this study as they are readily available to coaches/athletes/practitioners via commercial products.

Chapter 5

Effects of carbohydrate mouth rinse and caffeine on high-intensity interval running in a fed state

5.1 Introduction

Early CMR studies reported improvements in both running and cycling time trial performance (Carter *et al*., 2004; Pottier *et al*., 2010; Rollo *et al.,* 2008; Rollo & Williams., 2011). Carter *et al.,* (2004) investigated the effects of a 6.4% maltodextrin solution on a 1-hour time trial in comparison to a 0% solution. Improvements of 2.9% were reported for the average time to completion and the authors hypothesised that CMR's improvements in time trial performance is linked to the activation of the neural pathways via the oral receptors (Chambers *et al.,* 2009).

Whilst CMR studies have primarily been focused on cycling performance (Beelen *et al.,* 2009; Carter *et al.,* 2004; Chong *et al.,* 2011; Lesniak *et al.,* 2016), studies have shifted some focus to investigating the effects of CMR on other endurance sports (Dorling & Earnest., 2013; Kasper *et al.,* 2016; Rollo *et al.,* 2008; Rollo *et al.,* 2010). Following an overnight fast, Rollo *et al.,* (2008) investigated the effects of CMR on running performance with results showing a positive effect in relation to running speeds and total distance covered due to the CMR. Not all the findings from studies investigating CMR have been positive, as work by Beelen *et al* (2009) showed no enhancement in power output, heart rate or time to completion with a CMR (6.4% maltodextrin) whilst individuals were in a fed state. More recent work by Devenney et al (2016) demonstrated that when in a fed state, a 6% CMR (maltodextrin) can elicit a 5.6% performance improvement in cycling performance. With the effects of 6% and 6.4% solutions well documented, more recent studies have investigated higher concentrations CMR (Lane *et al.,* 2012; Devenney *et al.,* 2016). Improvements of 1.8% were reported in time trial performance with a 10% solution in a fed state, while improvements of 3% were reported in a fasted state (Lane *et al.,* 2012), while Devenney *et al* (2016) reported improvements of up to 6.1% with a 16% solution when compared to a 0% placebo. These findings compare favourably to previous studies of 6% and 6.4% solutions, showing increases in exercise performance linked to oral receptor activation (Chambers *et al.,* 2009; Frank *et al.,* 2008). These authors found that the use of CMR increased activation of the reward region of the brain, mainly the ventral striatum and anterior cingulate cortex (Chambers *et al.,* 2009; Frank *et al.,* 2008).

Caffeine ingestion (CAFF) can enhance repeated sprint performance in both running (Carr *et al.,* 2008; Mohr *et al.,* 2011) and cycling (Lee *et al.,* 2011). Kalmar (2005) reported that this endurance and sprint performance enhancement may be due to a mechanism of motor unit activity along with adenosine receptor antagonism. Recent work by Kasper *et al.* (2016) investigated the effects of CMR and the supplementation of caffeine on anaerobic running capacity in a glycogen depleted state. Participants completed a 45 minute steady state (65% $\dot{V}O_{2max}$) run followed by a HIT exercise capacity (1 minute at 80% $\dot{V}O_{2max}$ interspersed by one minute at 60% $\dot{V}O_{2max}$) until fatigue. The findings demonstrated an increased exercise capacity with carbohydrate mouth rinse and caffeine ingestion (CMR + CAFF) when compared to a placebo and CMR by itself. Other work by Kizzi *et al.* (2016) investigated the effects of a CAFF rinse compared to both a control (CON) and placebo (PLA) while participants were in a low endogenous CHO state. Although the study showed that peak power output was greater in the CON, CAFF rinse prevented a drop-off in power outputs across a repeated cycling sprint protocol in comparison to both the CON and PLA. Whilst the majority of the previously mentioned research has been carried out when athletes are in a fasted state, there is a greater need for research findings demonstrating the benefits of CMR in a fed state, as it is rare that athletes will endure competition in a fasted state. Although recent work by Lane *et al.* (2012) and Devenney *et al.* (2016) has begun to show the benefits of CMR in a fed state, more detailed work in the area is needed. The aim of this study was to ascertain whether CMR, either alone or in combination with caffeine, could enhance the high intensity running performance of recreationally trained athletes in a fed state, when compared to a placebo.

5.2 Methods

Recruitment: Eight recreationally trained males with a running background volunteered to participate in this study (age: 23 ± 3 years, body mass: 78 ± 7 kg, height: 1.75 ± 0.05 m, body-mass index: 22 ± 1.7 kg/m^2, $\dot{V}O_{2max}$: 51 ± 3 mL·kg^{-1}·min^{-1}), all of whom had engaged in exercise of 5-8 hours per week. All participants were informed of the study both verbally and in written form along with an outline of the physiological demands for each testing session. Medical questionnaire and consent forms were completed for each participant prior to testing. The study was approved by the local Research Ethics Committee.

Overall Study Design: In a randomised, repeated measures and double blinded study participants undertook a steady state run (45 minutes at 65% peak treadmill velocity, or PTV) followed by a HIT protocol until exhaustion (1-minute bouts at 90% PTV, separated by a 1-minute bout of walking at 6 km·h^{-1}). Participants consumed a standardised caffeine capsule (200mg) or capsulated placebo prior to the steady state running, and a second caffeine or placebo capsule was consumed prior to the commencement of the HIT protocol. Like CMR, a fixed dosage of caffeine (200mg) was utilised in the study rather than an optimal dosage (3-5mg/kg of BW) as from a practicality standpoint, athletes have this fixed dosage readily available to them via commercial products (gums, gels, shots, tablets). Throughout the HIT protocol participants rinsed with a 6% carbohydrate (maltodextrin) solution for 5 seconds on completion of every second interval. Therefore, each participant undertook three experimental trials which consisted of placebo capsule and placebo mouth rinse (PLA), placebo capsule and carbohydrate mouth rinse (CMR) and caffeine capsule and carbohydrate mouth rinse (CMR + CAFF). The primary marker during this study was time to fatigue in the HIT protocol. Heart rate (HR), blood lactate and rate of perceived exertion were monitored throughout the steady state run, while heart rate responses were monitored throughout the HIT protocol. Figure 1 gives an overview of the experimental design.

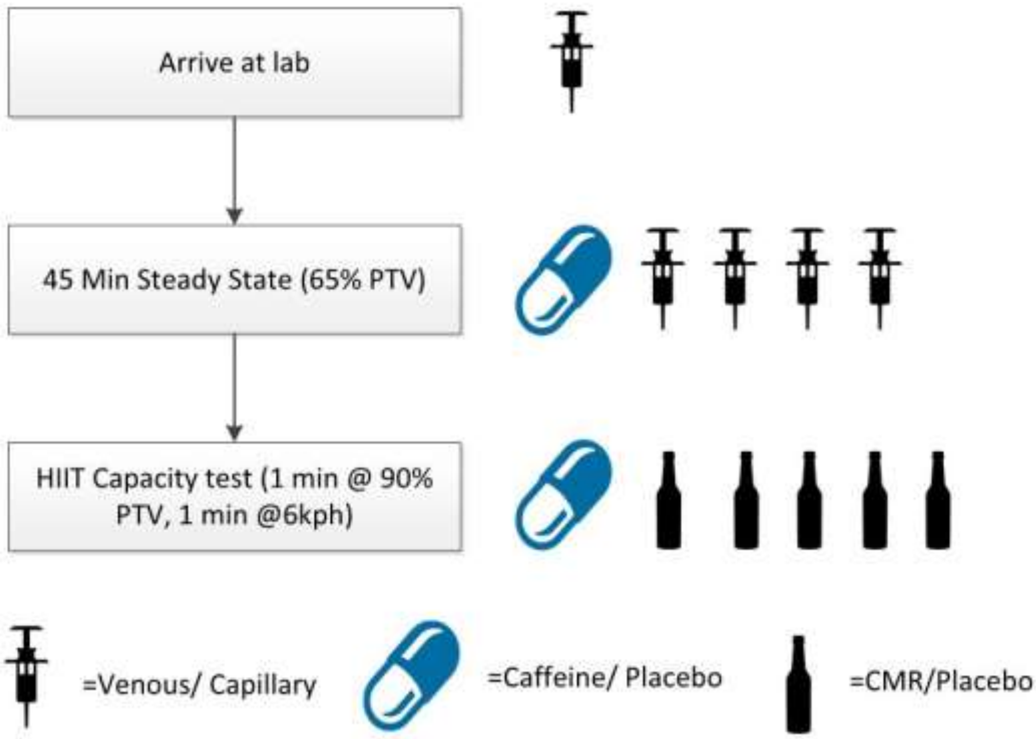


Figure 5.1: Schematic overview of the testing session.

Assessment of maximal oxygen uptake: A week prior to their first trial, each participant completed a graded exercise test on a motorised treadmill (Cosmed T170, Roma, Italy) to determine maximal oxygen uptake ($\dot{V}O_{2max}$). The protocol used was based on that of Akubat *et al.* (2014), with 4-minute stages interspersed with a minute recovery. Stages started at 8 km/hr with increments of 2 km/hr after completion of each stage (8, 10, 12, 14, 16 $km \cdot h^{-1}$) until onset of blood lactate accumulation

(OBLA) which was followed by a RAMP protocol (increments of 1 km·h^{-1}·min) to volatile exhaustion. Breath by breath analysis was recorded throughout the test using the Cosmed Quark CPET Metabolic Cart, Gas Analyser (Cosmed, Roma, Italy) with achievement of $\dot{V}O_{2max}$ determined by plateau of oxygen consumption despite an increase of workload and a RER value greater than 1.1. Running speeds for the experimental trials were determined from peak treadmill velocity (PTV). Post GXT, participants undertook a familiarisation process for the speeds at both the steady state (65% PTV) and HIT (90% PTV), so participants could familiarize themselves to the demands required in the remaining three visits.

Steady-state exercise protocol and HIT protocol: Kasper *et al.* (2016) implemented a 45 min steady state and chose to administer caffeine prior to the SS based on findings from Graham & Spriet (1995) which demonstrated that peak plasma caffeine typically occurs 45-minutes post ingestion. Therefore, this study implemented a similar steady state of 45 minutes set at 65% PTV for each participant. Participants arrived at the laboratory at their allocated time were given either 200 mg of caffeine (Bulk Powders, Essex, UK) or a visually identical placebo (Whey Protein Isolate, Bulk Powders, Essex, UK), prior to, and immediately after, the completion of the SS exercise protocol. The second caffeine capsule was administered prior to the HIT protocol as a means of further developing the ergogenic effect. Heart rate, levels of perceived effort (RPE) (Borg 1982; Borg 1987) and blood lactate were monitored throughout the SS exercise at time points of 5, 10, 20, 30, 40 and 45 minutes. Following the SS protocol, participants commenced a HIT protocol which consisted of 1-minute bouts at 90% PTV followed by a 1-minute bout of walking at 6 km·h^{-1} until physical exhaustion. Time to fatigue was calculated at the time which the participant could no longer meet the demands of the protocol. This follows similar methodology of prescribing interval where specific velocities are used to prescribe intervals (Buchheit & Laursen, 2013) Participants rinsed a 25 ml bolus containing either a 6% carbohydrate beverage

(Maltodextrin, Bulk Powders, Essex, UK) or a taste matched (orange) and visually identical placebo (Robinson Squash, Britvic Orange Soft Drinks, Hertsfordshire, UK) for 5 seconds periods every 4 minutes during exercise before expectorating.

Activity and Diet before Experiments: A two-day training diary was kept by each participant prior to each test visit, with low intensity exercise (heart rate below 150 beats/min) permitted for up to 60 minutes the day prior to testing. Alcohol and caffeine consumption was not permitted in the 24 hours prior to each visit. Dietary intake was recorded and was replicated for each visit as a measure to ensure repeatability. Participants consumed a mixed meal (eg. Meals recommended were a chicken and pasta with tomato sauce or a spaghetti Bolognese) 2-3 hours before testing (49 ± 2% carbohydrates, 18 ± 1 % protein, and 33 ± 2% fat) with each meal recommended to the participants by the authors. Although two-day dietary diaries were recorded and meals were recommended to each participant, the authors cannot guarantee participants adhered to these guidelines.

*Statistics***:** Data collected were analysed using a one-way repeated measures ANOVA (version 24 for Windows, IBM, SPSS). Before running the required ANOVA to investigate differences in exercise capacity (distance run during HIIT protocol) we performed a Shapiro-Wilks test to assess the data for normality. In the event that any statistically significant differences arose, a post hoc analysis was conducted comprising of a paired t-test with a Bonferonni adjustment of the level of alpha for multiple comparisons. Values are expressed as the means ± standard deviation with significance set at $p<0.05$. Effect size (ES) and 95% confidence (95% CI) intervals are reported for applicability to the general population. ES are reported in line with previous recommendations (Cohen, 1992; Lakens, 2013), using thresholds of 0.2 (small), 0.6 (moderate), and 1.2 (large) (Hopkins et al. 2009) and effects of 0.19 or less deemed trivial.

5.3 Results

Distance to Fatigue: In relation to running performance there was a significant increase in distance covered between the PLA and the CMR + CAF (p = 0.001, 95% CI: 1036, 2645 m; Cohens *d* = 1.34), and CMR and CMR + CAF (*p* = 0.031, 95% CI: 131, 2367 m; Cohens *d* = 0.87). However, there was no significant difference reported between the PLA and CMR (*p* = 0.218, 95% CI: -302, 1484 m; Cohens *d* = 0.46) although effect size can indicate a possible benefit. Mean distance covered across the individual trials were as followed; PLA, 4535 ± 1217 (3634, 5438) m; CMR, 5127 ± 1367 (4114, 6140) m; CMR + CAF, 6376 ± 1508 (5259, 7493) m and are illustrated in Figure 2.

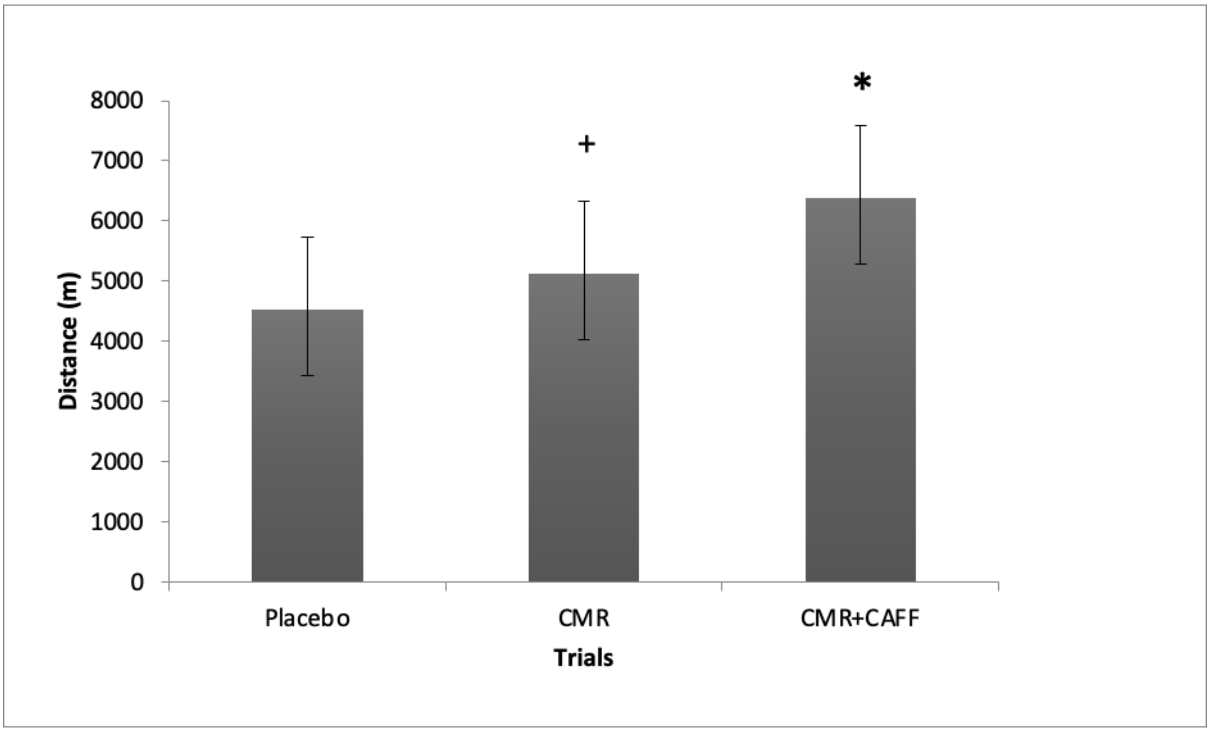


Figure 5.2: Mean distance to fatigue during the HIIT exercise test across all experimental trials. + denotes a possible benefit compared to the PLA and * denoted a significant benefit compared to the PLA.

Physiological Responses: Values for heart rate were seen to increase steadily with the onset of exercise across the three trials with Table 1 illustrating HR responses at set time points. There was no significant difference in heart rate responses across the three trials ($p > 0.05$).

Table 5.1 - Mean heart rate, blood lactate and RPE values across the three experimental trials. Average heart rate values are portrayed as beats per minute and blood lactate values are portrayed as mmol per litre.

		Time (Min)						
		5	10	20	30	40	45	Exhaustion
Placebo	Heart Rate ($b \cdot min^{-1}$)	155 ± 15	162 ± 14	167 ± 17	171 ± 13	171 ± 15	172 ± 15	188 ± 12
	Lactate ($mmol.L^{-1}$)	2.2 ± 0.9	2.3 ± 1.0	2.5 ± 1.1	2.5 ± 1.2	2.5 ± 1.1	2.4 ± 1.2	9.8 ± 2.3
	RPE (Borg Scale)	10 ± 2	11 ± 3	12 ± 2	14 ± 3	14 ± 2	14 ± 2	20 ± 1
CMR	Heart Rate ($b \cdot min^{-1}$)	151 ± 10	160 ± 11	164 ± 10	169 ± 12	169 ± 12	170 ± 13	188 ± 10
	Lactate ($mmol.L^{-1}$)	1.9 ± 0.6	2.1 ± 0.9	2.1 ± 0.9	2.1 ± 1.1	2.1 ± 1.1	2.2 ± 1.1	9.5 ± 3.0
	RPE (Borg Scale)	9 ± 2	9 ± 3	11 ± 2	13 ± 2	14 ± 2	13 ± 3	20 ± 1
CMR + CAFF	Heart Rate ($b \cdot min^{-1}$)	150 ± 12	160 ± 17	168 ± 16	171 ± 17	173 ± 16	174 ± 16	192 ± 10
	Lactate ($mmol.L^{-1}$)	1.9 ± 0.9	2.0 ± 0.9	2.4 ± 1.1	2.5 ± 1.0	2.6 ± 1.0	2.3 ± 1.1	10.1 ± 2.4
	RPE (Borg Scale)	9 ± 2	10 ± 2	11 ± 1	12 ± 3	13 ± 3	13 ± 2	20 ± 1

Similarly to HR, lactate responses remained constant throughout the steady state running, while an increase was seen post HIT across all three trials. No significance was observed when comparing lactate responses between the experimental trials ($p > 0.05$). Again similarly to HR and blood lactate, no significance was reported in RPE scores across all trials ($p > 0.05$)

Rinse solution detection: None of the participants involved in the study were able to differentiate between the mouth rinsing solutions used across the three experimental trials. Each participant was unable to distinguish any difference in taste or viscosity of the solutions.

5.4 Discussion

The aim of the study was to ascertain whether CMR, either alone or in addition to caffeine supplementation, could augment high intensity running performance in comparison to a placebo in recreationally trained males in a fed state. The findings from the study support the initial hypothesis of incremental benefit to running performance, similar to that of previous work by Kasper *et al.* (2016). Although the statistical certainty around the improvements in the CMR group are not as strong ($p = 0.218$) in relation to distance to fatigue, the CMR does illicit a small to moderate effect (Cohens $d = 0.46$). We also confirm that caffeine maintains its ergogenic effects despite exercising in a fed state.

Previous work examining the effect of CMR in a post prandial state has been somewhat equivocal. Early work revealed no significant effect of CMR on exercise performance in a post prandial state (Beelen *et al.*, 2009; Whitham & McKinney, 2007) while more recent work has shown performance improvements of 1.8-7.1% (Devenney *et al.*, 2016; Lane *et al.*, 2012) albeit with higher solution concentrations in conjunction to the more traditional CMR concentrations. While the CMR did not produce a statistically significant benefit, the difference confidence intervals and moderate Cohen's *d* effect size would suggest that there is still a benefit elicited from the CMR condition. This would support previous work which has demonstrated that while there seems to be greater benefit in a fasted state, a benefit is still obtained from CMR even in the fed state (Devenney *et al.*, 2016; Fares & Kayser, 2011; Lane *et al.*, 2013).

In a bid to further enhance exercise performance, caffeine capsules were consumed both pre and post steady state sessions to allow peak plasma levels before the commencement of the HIT protocol (Goldstein *et al.*, 2010). Compared to a placebo, this addition of caffeine had a profound effect on running distance with significant difference in distance to fatigue and a large effect size (Cohen's d = 1.34). This would indicate strong merit in the prescription of CMR + CAFF for those undertaking high intensity running. Furthermore, not only did the CMR + CAFF condition have a significant benefit over the placebo condition, but a significant increase in running distance when compared to the CMR only condition was also observed. Thus, it seems the addition of caffeine exerts a moderate effect (Cohen's d = 0.87) on high intensity running performance. This study, alongside the work of Kasper *et al,* (2016), provides compelling evidence for the addition of caffeine to CMR to further augment performance. CMR is believed to stimulate the reward pathways of the brain via oral receptors due to a carbohydrate solutions caloric content (Chambers *et al*., 2009; Turner *et al.,* 2014). One would envision that this stimulation would illicit lower RPE levels in the CMR trials compared to the PLA, leading to a viable reason for the increase in exercise performance, as exercise intensity or tolerance can increase to compensate for the drop in RPE levels. However, previous research has reported a lack of a difference in RPE and HR responses (Beaven *et al.,* 2013; Carter *et al.,* 2004; Chambers *et al.,* 2009; Devenney *et al.,* 2016; Pottier *et al.,* 2010) and the current study confirms the findings, with no significant difference reported in RPE or HR levels between either of the CMR or CMR + CAFF trials and the PLA trial.

There were limitations to consider within the current study. As participants were recreationally trained athletes, it is unknown if the current results can translate to the well-trained athletic population. An additional limitation of this study is the sample size (n=8), therefore the authors utilised effect size to understand the practical significance of the results. A further limitation to the study is the exclusion of a CAFF only group, as it is unsure what benefit CAFF supplementation on its own would have throughout the time to fatigue protocol. As the primary focus of the study was CMR, whether alone or with CAFF, would improve exercise performance, the inclusion of a CAFF trial could have further strengthened the findings of the research. Although practical significance was found, the authors issue caution due to the small sample size. Although subjects were reported low caffeine consumers, caffeine habituation was not measured and therefore may have affected acute responses to the caffeine dose. There was no utilization of a non-rinse control, nor was the possibility of a dose–response addressed.

5.5 Conclusion

In conclusion, the study provides novel data which illustrates that CMR, whether alone or in tangent with caffeine supplementation, can delay the onset of fatigue in anaerobic interval running. With the benefits already reported, these findings along with previous research suggest that the regular use of CMR can decrease the risk of gastrointestinal distress reported by athletes, meaning the data can be applicable to both athletes and coaches in a real-world setting.

Key Findings

Results indicate that CMR+CAFF (6% CHO + 200mg) significantly improve distance to fatigue, when compared PLA and CMR, and although no significant difference was observed when comparing CMR to PLA, results do intake a potential benefit to performance (Cohens *d* = 0.46).

Link to Next Experimental Chapter

With the previous research showing the benefits of CMR+CAFF and the potential benefit of CMR to running performance in a practical environment (i.e., in a fed state), the authors wanted to build on the foundations laid by the study by Kizzi et al., (2016) and determine the effects of CHO and/or CAFF on repeated cycling performance. This study compared a CAFF rinse to a PLA and non-rinse CONT, so authors used these conditions with the addition of a CMR condition & a CMR + CAFF rinse condition. With CHO and CAFF two heavily reliant supplements for sports performance and research showing a potential benefit on PPO in a fasted state, the study again applies a more practically applicable setting with participants in a fed state. Participant recruitment again switched to recreational athletes but did not require a cycling or running background. A CAFF rinse was utilised in this study as it is the main focus of the study by Kizzi et al., (2016) but also to determine whether there is any potential benefit when compared to a CMR or CMR+CAFF.

Chapter 6

Effects of carbohydrate and caffeine mouth rinse on repeated sprint performance in a fed state

6.1 Introduction

The benefits of carbohydrate ingestion on exercise performance has been well documented for both prolonged (>2hours) and high intensity (75% $\dot{V}O_{2max}$) bouts of exercise (Coyle *et al.,* 1986:, Foskett *et al.,* 2008), as it reduces the rate of liver glycogen depletion and slows the fatty acid utilisation rates (Hargreaves *et al.,* 2004:, Jeukendrup *et al.,* 1999:, Jeukendrup *et al.,* 2000:, Stellingwreff *et al.,* 2007:, Van Loon *et al.,* 1999:, Tsintzas *et al.,* 1998). More recent studies have shown that exogenous carbohydrates supplementation in the form of CMR may improve exercise performance, with the earliest work by Carter et al., (2004) showing improvements of 2.9% in relation to cycling time trial performance. It is hypothesized that this performance improvement can be related to the CMR stimulating oral receptors linked activation of the central nervous system (Chambers *et al.*, 2008).

Follow-up studies to that of Carter et al., (2004) have investigated the effects of CMR on other endurance sports (Devenney et al. 2018; Germaine et al. 2018; Dorling *et al.*, 2013:, Kasper *et al.,* 2015:, Rollo *et al.,* 2008:, Rollo *et al.,* 2010), with Rollo et al., (2008) showing that CMR has positive benefits to running performance in relation to running speeds and total distance covered. With much of the aforementioned work looking at the benefits of CMR in a fasted state, work by Beelen et al (2009) showed no benefits performance with CMR (6.4% maltodextrin) while in a fed state, citing no difference in the time to completion, heart rate or power output. Improvements of 5.6% have been reported by Devenney et al (2016) for cycling time trial performance with the use of CMR (6% solution) while in a fed state.

The benefits of caffeine ingestion on repeated sprint performance for both running (Carr et al. 2008; Germaine et al. 2018; Mohr et al. 2011) and cycling (Lee et al. 2011) are well documented, with Kalmar (2005) reporting that enhancement in endurance and sprint performance may be due to a mechanism of the motor unit activity along with the adenosine receptor antagonism. With the initial results of CMR showing beneficial improvements, the impact of caffeine mouth rinse (CAFF) was then explored. Beaven et al (2013) compared the effects of CMR and CAFF in repeated sprints with recreationally trained males, showing that although no significance was observed in relation to HR and RPE, CMR increased peak power output (PPO) of sprint 1 and both CMR and CAFF trials increased mean power output of sprint 1 when compared to a placebo (ES 0.81-1.08). The authors also reported further enhancements in PPO when utilising a combo of CMR+CAFF when compared to just CMR. Studies by Kizzi et al (2016) and Lesniak et al (2016) have continued looking at the effects of caffeine rinse on cycling performance, looking at repeated sprints and cycling TT performance respectively. Kizzi et al (2016) reported that CAFF reduces the performance decrement of power output in repeated sprints whilst findings from Lesniak et al (2016) showed no significance in CMR, CAFF or CMR+CAFF in cycling endurance performance in female athletes.

Therefore, the aim of this present study was to investigate whether carbohydrate mouth rinsing, caffeine rinse or a carbohydrate/caffeine rinse could augment repeated sprint performance when compared to a placebo and non-rinse control in a fed state in recreationally trained males.

6.2 Methods

Subjects: Fourteen recreationally active males (aged 22 ± 3 yrs, body mass 71.5 ± 10.1 kg, stature 173.5 ± 7.6 cm, skinfolds 75.6 ± 19.3 mm, maximal power output [W_{max}] 305 ± 30W, $\dot{V}O_{2max}$ 52.7 ± 5.8 $ml \cdot kg^{-1} \cdot min^{-1}$) participated in this randomised, double blind, and repeated measures crossover investigation. Participants involved gave written informed consent and ethics was approved by the local research ethics committee. All participants were free from medication, and abstained from exercise, alcohol and all caffeinated beverages for a total of 24h prior to testing.

Overall Study Design: Participants visited the laboratory on six separate occasions. On the first visit, athletes underwent anthropometric assessment and an incremental test to determine the $\dot{V}O_{2max}$ of the participant. Participants were also given a repeated sprint familiarisation trial on their second visit to the lab. Participants then attended the lab on four separate occasions for each repeated sprint protocol (six 10s sprint, each separated by 50s active recovery) each with a different mouth rinse trial (Figure 1).

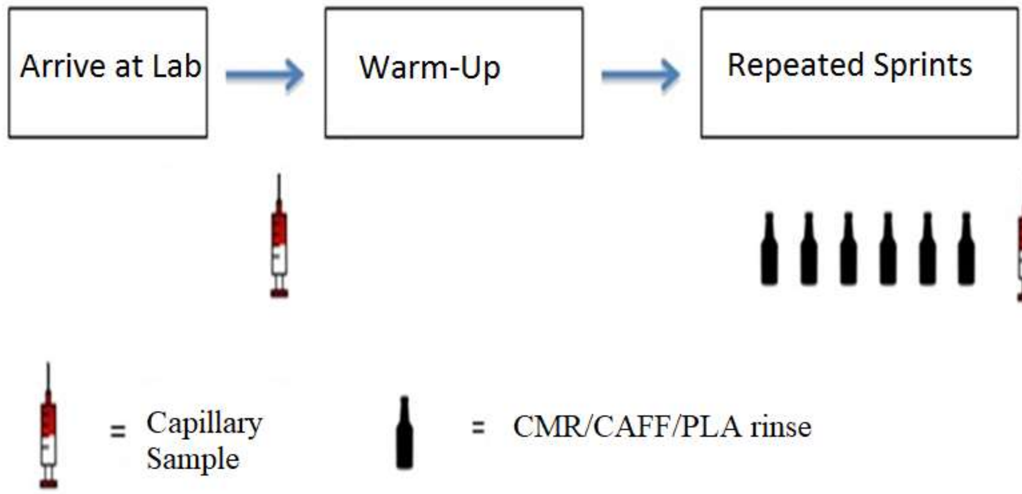


Figure 6.1: Schematic overview of the protocol design

Incremental Test: The incremental test was performed on a cycle ergometer (Wattbike Pro, British Cycling Wattbike, Nottingham, England) consisting of a 3 min warm up at 100 W, followed by increments of 30 W every 3 min, until voluntary exhaustion or when the participant were unable to maintain the required power output. Maximal power output (W_{max}) was defined as the highest power output maintained during a complete 3 min stage. When the last stage of the incremental test was not completed, W_{max} was determined in accordance with the precise methods of Kuipers, Verstappen, Keizer, Geurten and van Kranenburg (1985).

Activity and Diet before Experiments: Participants kept a two-day training diary prior to each test visit, and were allowed to undertake low intensity exercise (heart rate below 150 beats/min) for up to 2 hours the day prior to testing. Participants were asked to refrain from alcohol and caffeine consumption in the 24 hours prior to each visit. Dietary intake was recorded to prevent the disruption of the results and was replicated for each visit throughout the study. Participants consumed a meal 2-3 hours before testing (49 ± 2% carbohydrates, 18 ± 1 % protein, and 33 ± 2% fat) with each meal recommended to the participants by the authors.

Repeated sprint cycling test: During five separate visits to the lab, participants performed six, 10 s cycling sprints interspersed with 50 s active recovery under the following conditions: (1) control trial (CONT) that involved no mouth rinse acting as a baseline value for participants, (2) a placebo trial (PLA) that contained no supplementation within the rinse, (3) a CHO rinse (6% maltodextrin), (4) a CAFF rinse (1.2% caffeine), and (5) a CHO and CAFF rinse (6% maltodextrin and 1.2% caffeine). Randomisation of trials was ensured by assigning each mouth rinse condition a number (2-5), using a computer program (Research randomiser: Version 4.0) which generated fourteen sets of randomised trials. Each visit to the lab was separated by five days for washout. Although CAFF has a half like of 5-6hrs and effects would be elapsed after 24hrs, a 5-day washout period was utilised to replicate a weekly training cycle that athletes would typically adhere to. Prior to commencement of each testing protocol, baseline blood lactate values were recorded from the ear lobe using a lactate scout (Nova, Biomedical, USA) with each participant fitted with a heart rate monitor (Polar T31, Polar Electro, Tampere, Finland) which was linked to the Wattbike. For each trial, participants completed a standardised 3 min warm up at 100W on a cycle ergometer (Wattbike Pro, British Cycling Wattbike, Nottingham, England). After the 3 min warm up, visualisation on the screen was blinded for the particpant showing just the clock for time, with power, speed and cadence invisable to the eye of the participant. During the 50 s active recovery in the lead up to the sprint, particiants were administered with a mouth rinse solution for 5 s, before expectorating into a waste container. Participants rinsed for six times in total prior to each 10 s sprint.with the rinse occuring during each active recovery session. Participants were required to cycle at what they percieved to be 50 W during the active recovery session with a verbal countdown to start in five, 10s maximal sprint efforts with air resistance set at 4 due to body mass guidelines set out by the manufacture of the wattbike. All variables from the testing procedure were recorded on a laptop which contained the wattbike software. Throughout each maximal sprint, no verbal encouragement was given to any participant and the only interaction was during administeration of solutions and with the countdown to the start and end of each sprint.

Participants were asked to provide a rating for percieved exertion (Borg, 1982, 1990) at the end of each sprint, a visual scale was present for each test for visulaisation and more accurate values. Familarisation with the scale was provided during the control trial with no mouth rinse and also at the start of each testing procedure. A blood lactate value was also taken at the end of each sprint from the ear lobe once again.

Mouth Rinse Protocol: Over the course of the six visits to the lab, participants would be administered four different mouth rinses along with the non rinse control, a placebo trial (PLA) that contained no supplementation within the rinse, a CHO rinse (6% maltodextrin), a CAFF rinse (1.2% caffeine), and a CHO and CAFF rinse (6% maltodextrin and 1.2% caffeine). Each sample was a 25ml bolus which was weighed before and after the mouth rinsing to ensure that none of the sample was ingested. The solution was supplied to the participant after the warmup session and prior to the sprint (during the 50 s active recovery). Each solution was rinsed in the mouth for 5 s before being expelled into a pre weighed container. All solutions utilised in the testing procedure were made by an external researcher who was not affiliated to the study. This was conducted to ensure the investigation remained a double-blind study.

Statistical Analysis:

Data were analysed using SPSS Statistics version 23 (IBM North America, New York, NY, USA). To determine parametricity, Levene’s tests (homogeneity of variance) and Shapiro–Wilk (normal distribution) were employed. Significance was set at $P < .05$ and ESs are reported for primary outcome measures in line with previous recommendations (Cohen, 1992; Lakens, 2013) using thresholds of 0.2, 0.6, and 1.2 for small, moderate, and large, respectively (Hopkins et al. 2009) and effects of between –0.19 and 0.19 deemed trivial.

6.3 Results

Peak Power Output: Although no significant difference was reported across trials ($p > 0.05$) results indicate that in relation to peak power output of sprint 1, there is a small effect when using the CHO (Cohen's d, 0.40; small), CAFF (Cohen's d, 0.45; small) or CHO/CAFF (Cohen's d, 0.53; small to moderate) versus the CONT. The CHO/CAFF trial also elicited a small response when compared to the PLA trial (Cohen's d, 0.3; small). Similarly for the final sprint, there is a small effect when using the CHO (Cohen's d, 0.23; small), CAFF (Cohen's d, 0.28; small), or CHO/CAFF rinse (Cohen's d, 0.28; small), in comparison to the PLA and CONT. No variation was observed when comparing the CHO to either the CAFF or CHO/CAFF.

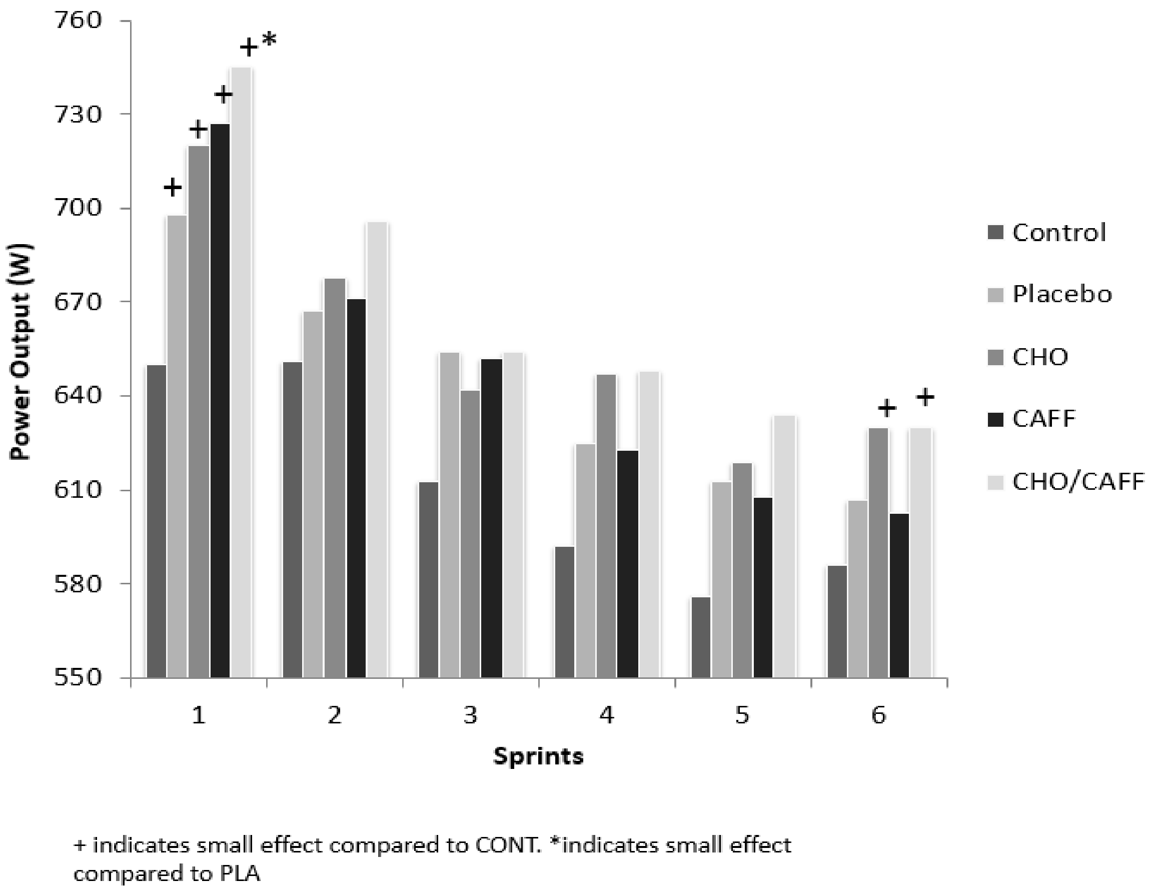


Figure 6.2: Power output responses across all 5 trial conditions.

Physiological Responses: Values for heart rate were seen to increase steadily with the onset of exercise across the five trials with Table 1 illustrating HR responses after each sprint. There was no significant difference in heart rate responses across any of the five trials ($p > 0.05$).

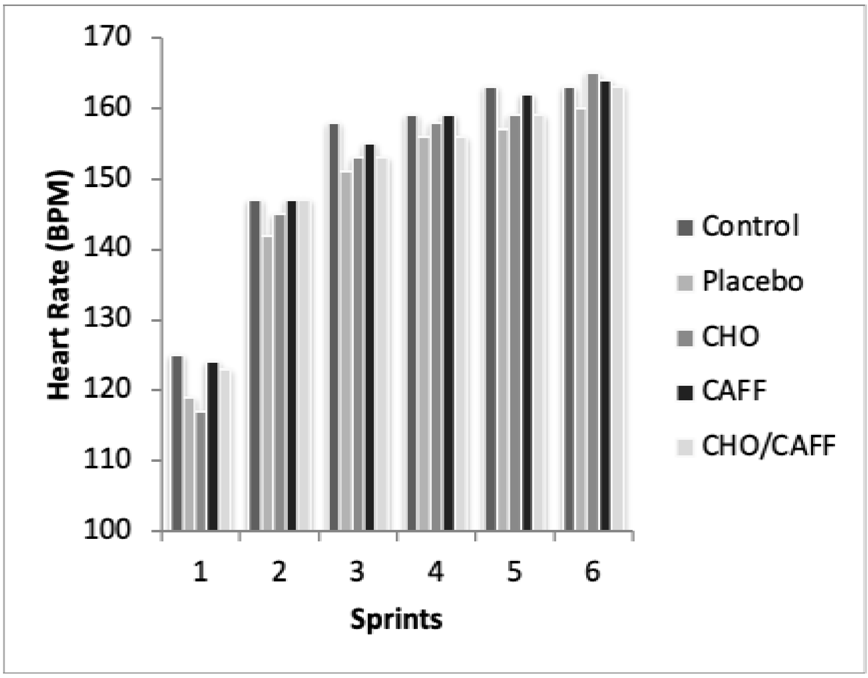


Figure 6.3: Mean heart rate responses across all 5 trial conditions.

Similarly, to HR, RPE responses increased steadily after each of the 6 sprints across all five trials, with Figure 4 illustrating RPE values after each sprint. There was no significant difference in RPE values across any of the five trials ($p > 0.05$) No significance was observed when comparing pre and post lactate responses between the experimental trials ($p > 0.05$).

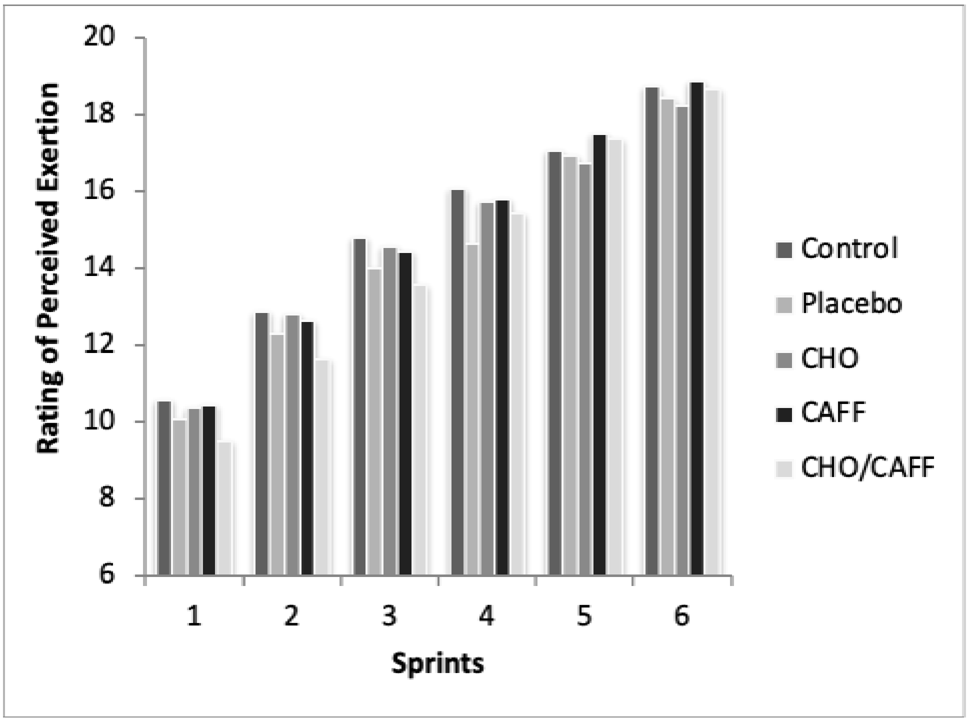


Figure 6.4: **Mean RPE responses across all 5 trial conditions**

Rinse solution detection: It was noted that one of the participants was able to distinguish a taste difference between the CHO trial and the CAFF and CHO/CAFF trials. However, the participant was unable to distinguish any difference in taste between the CAFF and CHO/CAFF trial. The remainder of the cohort were unable to differentiate between the mouth rinsing solutions used across the five experimental trials. Each participant was unable to distinguish any difference in taste or viscosity of the solutions.

6.4 Discussion

The aim of the study was to investigate whether carbohydrate mouth rinsing, caffeine rinse or a combination, could augment repeated cycling sprint performance when compared to a placebo and a non-rinse control in recreationally trained males in a fed state. The main findings of this investigation support previous findings by Beaven et al (2013) and Kizzi et al (2016), that carbohydrate and/or caffeine mouth rinse can enhance initial cycling sprint power output in recreational athletes. Although the statistical certainty around the improvements in the CMR group are not as strong ($p > 0.05$) in relation to peak power output, the CMR does illicit a small to moderate effect (Cohens $d = 0.46 – 0.53$). We also confirm that caffeine maintains its ergogenic effects despite exercising in a fed state.

The effect of CMR in a post prandial state has been somewhat equivocal, with early work stating no significant effect for CMR (Beelen *et al.*, 2009; Whitham & McKinney, 2007) but more recent work reporting improvements of 1.8-7.1% for performance markers (Devenney *et al.,* 2016; Lane *et al.,* 2012) albeit with higher solution concentrations. While the CMR did not produce a statistically significant benefit, a small Cohen's *d* effect size would suggest that there is still a benefit elicited from the CMR condition. This would support previous work which has demonstrated that while there seems to be greater benefit in a fasted state, a benefit is still obtained from CMR even in the fed state (Devenney *et al.,* 2016; Fares & Kayser, 2011; Lane *et al.,* 2013).

In a bid to further enhance exercise performance, caffeine supplementation has been shown to improve both endurance and sprint performance (Carr et al 2008, Germaine et al 2019, Kalmar 2005, Lee et al 2011, Mohr et al 2011). While previous literature has investigated the effects of caffeine ingestion and its effects on exercise performance, the current study applies a novel method of delivery along with concentration, in the form of 1.2% w/v mouth rinse. Like CMR, although the CAFF condition did not produce a statistically significant benefit, a small Cohen's *d* effect size would again suggest a benefit. Furthermore, the combination of CMR+CAFF not only demonstrated a marginal performance benefit in relation to the CONT but also in relation to the PLA. This study, alongside the work of Beaven *et al,* (2013) and Kizzi *et al*, (2016), provides compelling evidence for the addition of caffeine to CMR to further augment performance.

It is well documented that CMR is believed to activate the reward pathways of the brain, in particular the ventral striatum and anterior cingulate cortex, via oral receptors due to the solutions caloric content (Chambers *et al*., 2009; Turner *et al.,* 2014). This stimulation should illicit lower RPE levels in the CMR, CAFF and CMR/CAFF trials compared to both the PLA and CONT, leading to a plausible rationale for the increase in power output, as exercise intensity can increase to compensate for the drop in RPE levels. However, no difference has reported in RPE levels responses, which is also the case in previous literature (Beaven *et al.,* 2013; Carter *et al.,* 2004; Chambers *et al.*, 2009; Devenney *et al.,* 2016; Pottier *et al.,* 2010). Similarly with HR values, there was no significant difference reported for values between the three supplement trials (CMR, CAFF and CMR/CAFF) and the both the PLA and CONT trial, again which follows the trend report by previous research. Beaven et al (2013) hypothesised that the lack of difference in both HR and RPE values could be down to the maximal nature of the sprint, which would illicit maximal response with RPE and HR responses, which would lend to this study.

There are a number of limitations to the current study, the first being the training nature of the participant pool. The participants used in the study were recreationally trained and therefore it is unknown if the results of this study will translate to the highly trained athletic population. The second limitation was that caffeine habituation was not monitored in this study and, although subjects reported as being low caffeine consumers, it may have affected the acute dose response to the caffeine.

6.5 Conclusion

In conclusion, the present study shows that the use carbohydrate and/or caffeine mouth rinsing during repeated cycling sprints can lead to an improvement in power output and exercise capacity when exercise is carried out in a fed state. With the ergogenic benefits well documented, the data can be applied to the practical setting for athletes and coaches alike, as the findings suggest that CMR at regular intervals can further enhance performance markers and allow for greater training adaptations along with benefitting athletes reporting gastrointestinal problems.

Chapter 7

Synthesis of Findings

7. Synthesis of Findings

This current chapter provides an overview of the findings of the aims and objectives which was set out in Chapter 1. A general discussion is also presented which gives specific details to how the current data gives advances in the benefits of CMR in relation to exercise performance. Finally, this chapter also outlines some limitations that were encountered throughout the process of these studies, along with identifying the practical applications of the findings and some potential avenues for future research.

7.1 Achievements of Aims and Objectives

The aim of this thesis was to determine if CMR can positively impact exercise performance when athletes are in a fed state across of multitude of protocols. Although some findings of previous research are conflicting, the positive impacts of CMR on exercise performance are well reported when athletes were in a fasted/glycogen depleted state, therefore this current work aims to build on these foundations and determine if there is a potential benefit when athletes are in a practically applicable state. These aims were to be achieved via the completion of three objectives/studies highlighted in Chapter 1 and which will each be discussed.

Aim 1

Determining the effects various concentrations of CMR has on cycling time trial performance while athletes are in a fed state (Study 1).

This aim was investigated in Study 1 (Chapter 4). For this thesis to add further value to the current literature findings it mirrored the study by Beelen and colleagues (2009), looking to see if a 6% CMR could improve exercise performance. Furthermore, the study aimed to see if further saturation of the oral receptors via a 16% CMR could improve exercise performance. Participants undertook three trial conditions (PLA, 6%, 16%) in a simulated TT outlined in Chapter 4. Although no significant differences in participant RPE or HR levels were reported, time to completion in the cycling protocol improved by 5.6% for the 6% CMR and 7.1% for the 16% CMR when compared to the PLA condition. These findings contradict the findings by Beelen et al. (2009) and provide a platform by which CMR can improve exercise performance when participants are in a fed state.

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Aim 2

Determining the effects of CMR and caffeine supplementation has on steady state running and high intensity intervals (Study 2).

The aim was addressed in Study 2 (Chapter 5). With the benefits of CMR established in Study 1 and with caffeine supplementation becoming more popular in the sporting world, this study aimed to provide evidence of a potential ergonomic benefit of CMR with CAFF in SS running and HIIT. Similar to Study 1, no significant differences were reported for participant RPE, HR or blood lactate for the CMR & CMR+CAFF trials when compared to the PLA. CMR+CAFF elicit a large ES (Cohens d = 1.34) when compared to the PLA and a moderate ES (Cohens d = 0.87) when compared to CMR alone, due to a superior distance covered in the HIIT protocol. Although no significance was observed when comparing CMR to PLA ($P > 0.05$), a potential benefit can be observed due to ES (Cohen's d = 0.46). Again, these finds detail how CMR and CMR+CAFF can improve exercise performance and therefore potentially enhance training adaptations like to HIIT.

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Aim 3

Determining the effects of CMR and CAFF on repeated cycling sprints while in a fed state (Study 3).

This aim was addressed in Study 3 (Chapter 6). With Study 2 showing the positive impact CMR and CAFF supplementation can have on have on exercise performance, focused shifted to determine if CAFF rinse could impact exercise performance, either standalone or with a CMR combination. The study used a RSA which is outlined in Chapter 6 and compared a 6% CMR, 1.2% CAFF and 6% CMR+1.2% CAFF trial to a PLA and non-rinse CONT. Although no significant difference was reported in PPO in each sprint when comparing trials, small ES (Cohen's d = 0.40-0.53) was reported for CMR, CAFF & CMR+CAFF versus the PLA and CONT for Sprint 1, and small ES (Cohen's d = 0.23-0.25) for Sprint 6 meaning a marginal benefit in relation to PPO. Furthermore, the combination of CMR+CAFF not only demonstrated a marginal/trivial benefit to exercise performance compared to the CONT but also when compared to PLA.

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7.2 General Discussion

Since the initial study by Carter and colleagues (2004), research has significantly enhanced the findings and understandings of the performance benefits of CMR across a magnitude of sports. However, most of the findings have been associated with exercise in a fasted/low glycogen state with inconclusive data associated when exercise is carried out in a fed/post prandial state. This therefore makes the practical application to a training or race environment unknown, as athletes typically tend to train/race when substrate availability is at an optimal level. With early work stating no significant effect for CMR while in a post prandial state (Beelen *et al.*, 2009; Whitham & McKinney, 2007), this present thesis provided novel data for the literature by reporting improvements in exercise performance for cycling TT while in a fed state. By using a set workload that participants needed to complete and by monitoring physiological responses (HR, blood lactate and RPE), it was determined that CMR with either a 6% or 16% solution can improve time to completion by 5.6% and 7.1% respectively in comparison to a PLA. With no differences in physiological data across all three trials, the percentage improvements could be driven by the hypothesis of a central activation of reward pathways as outlaid by Chambers and colleagues (2009). Further improvements due to a higher concentration of solution could be due to a greater saturation of the oral receptors noted by Chambers et al. (2009) and Turner (2014), however further investigation on this is required.

In a bid to further add to the current literature, the addition of a CAFF supplementation to the CMR was investigated. It was hypothesized that the addition of caffeine to CMR could further augment high intensity running, a hypothesis which was previously investigate by Kasper and colleagues (2016) albeit in a fasted state. The current work aimed to provide a scientific foundation for the benefits of CMR and CAFF supplementation in a carbohydrate fed state, with the reported results showing a greater distance to fatigue in the CMR+CAFF trial when compared to the PLA and CMR, with ES of 1.34 and 0.87 (Cohen's *d*) reported. More recent work by Germaine and colleagues (2018) reported no significant difference when comparing CMR+CAFF to CAFF alone ($p = 0.99$), using the same protocol as this current study. Although there was no significant difference in distance to fatigue reported between CMR and PLA ($p = 0.218$), an ES of 0.46 (Cohen's *d*) was reported leading to a potential benefit elicited from the CMR. These findings would support previous literature which has demonstrated that whilst there is a greater greater benefit in a fasted state, a benefit is still obtained from CMR even in the fed state (Devenney et al. 2016; Fares and Kayser 2011; Lane et al. 2013). It could be envisioned that the stimulation via CMR would illicit lower RPE levels compared with the PLA trial, leading to a viable reason for the greater time to fatigue, as exercise intensity or tolerance can increase to compensate for the lower levels in RPE. However, previous research has reported a lack of a difference in RPE and HR responses (Beaven et al. 2013; Carter et al. 2004; Chambers et al. 2009; Devenney et al. 2016; Pottier et al. 2010).

In addition to caffeine supplementation, emerging data has shown potential benefits to caffeine rinse on exercise performance. In study 3, we employed a RSA (10s on/50s off) protocol comparing CMR, CMR+CAFF, CAFF to a PLA and a CONT, mirroring work already carried out by Beaven (2013) and Kizzi (2016) but with altered trials. Although the results across all trials were not significantly different ($p > 0.05$), the use of ES showed a small to moderate effect for PPO in Sprint 1 for the CMR, CMR+CAFF and CAFF trials compared to the PLA and CONT and a small effect in the final sprint, again with the CMR, CMR+CAFF and CAFF in comparison to both the PLA & CONT. Similar to the previous bodies of work no difference was reported in HR, RPE level or pre/post blood lactate levels, therefore leading to the hypothesis that the increase in performance is linked to the neurological pathway activation. With the link between CMR and oral receptors already well documented, this current body of work also hypothesizes that there could be similar oral receptors linked to CAFF and the reward pathways of the brain, but further work is required on this topic.

7.3 Limitations

7.3.1 Participant Population

The first limitation to this thesis was the participant population, as participants across all three studies were recreationally trained athletes. Although their athletic profiles (stated in Chapters 4-6) cover a far greater spectrum by which the performance benefits can be seen in a real-world setting, it is unknown whether the performance improvements reported above will translate to a sub elite or elite population for that matter.

7.3.2 Supplementation and Rinse Protocols

Another limitation to this thesis is the supplementation and rinse protocols. Although subjects were reported low caffeine consumers, caffeine habituation was not recorded which may have affected the acute responses to the caffeine dose. There was no utilization of a non-rinse control in Study 1 or 2, nor was the possibility of a dose–response addressed. Furthermore, some participants ability to detect a difference in taste across the rinse trials due to the nature of caffeine as a supplement and its unique taste.

7.4 Practical Implications

With the ergogenic benefits of CMR well documented in previous literature and with this current thesis, the data reported can be applied to the practical setting for athletes and coaches alike, as findings suggest that CMR at regular intervals can further enhance athletics performance and allow for greater training adaptations without impacting physiological markers (HR, blood lactate and RPE) along with benefitting athletes reporting gastrointestinal problems.

7.5 Future Research

With the benefits of CMR well documented now since the initial study by Carter and colleagues (2004), and studies looking at the neural activation pathways (Chambers et al, 2009 & Franks et al, 2010), further research is required to investigate the activations of the highlighted neural pathways whilst participants are exercise. This will highlight what the true cause of exercise improvements are in relation to CMR, and whether there is a substantial activation of the reward regions of the brain during exercise or is improvements are due to psychosomatic reasons. Furthermore, trials on highly athletic population are required to determine if the improvements reported for recreational athletes can translate to athletes with a superior physiological profile and training status.

Chapter 8

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Appendix

Appendix 1 - Devenney, S., Collins, K., & Shortall, M. (2016). Effects of various concentrations of carbohydrate mouth rinse on cycling performance in a fed state. European journal of sport science, 16(8), 1073-1078.

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Effects of various concentrations of carbohydrate mouth rinse on cycling performance in a fed state

Simon Devenney, Kieran Collins & Marcus Shortall

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Routledge

ORIGINAL ARTICLE

Effects of various concentrations of carbohydrate mouth rinse on cycling performance in a fed state

SIMON DEVENNEY, KIERAN COLLINS, & MARCUS SHORTALL

Department of Science, Centre for Exercise and Metabolic Science, Institute of Technology Tallaght, Dublin, Ireland

Abstract
The objective of this study was to identify the effects of mouth rinsing with a 6% and 16% carbohydrate solution (CHO) on time trial performance when compared to a 0% control (PLA) when in a fed state. Twelve recreationally active males underwent three trials by which they had to complete a set workload (600 ± 65 W) in a fed state. Throughout each trial, participants rinsed their mouths with a 25 ml bolus of a 0% PLA, 6% or 16% CHO (maltodextrin) for every 12.5% of work completed. Rating of perceived exertion (RPE) and heart rate were recorded every 12.5% of total work. Performance times and power output improved significantly when using the 6% and 16% CHO versus the PLA trial (6% versus PLA, $p = .002$ and 16% versus PLA, $p = .001$). When comparing the performance times of the 6% to 16% CHO, no significance was observed ($p = .244$). There was no significant difference between heart rate levels or RPE values across the three trials. In conclusion, mouth rinsing with a 6% or 16% CHO solution has a positive effect on a cycling time trial performance undertaken in a fed state.

Keywords: *Maltodextrin; 16% CHO solution; 6% CHO solution; exercise; power output; time trial performance*

Introduction

The ingestion of carbohydrates and their beneficial effects on exercise performance in both prolonged bouts of exercise (>2 h) and also in high-intensity exercise (>75% VO_2max) have been previously documented (Coyle, Coggan, Hemmert, & Ivy, 1986; Foskett, Williams, Boobis, & Tsintzas, 2008). Carbohydrate ingestion can have a positive impact during exercise as it plays an important role in reducing the rate of liver glycogen depletion and slows the rate by which fatty acids are utilised as a fuel source (Hargreaves, Hawley, & Jeukendrup, 2004; Jeukendrup et al., 1999; Jeukendrup, Rollo, & Carter, 2013; Stellingwerff et al., 2007; Tsintzas and Williams, 1998; van Loon, Jeukendrup, Saris, & Wagenmakers, 1999).

It has been reported that mouth rinsing with a carbohydrate solution (CHO) works best during the later stages of a 1 h cycling time trial, as there is a reduction in power (Carter, Jeukendrup, & Jones, 2004). Carter et al. (2004) investigated the effects of rinsing a participant's mouth with a CHO (6.4% maltodextrin) compared to a 0% placebo (PLA) during a 1 h time trial. Each solution was rinsed around the mouth every 12.5% of the trial completed and then expelled in order to prevent swallowing the solution. Results reported that the use of 6.4% CHO solution meant a 2.9% improvement in average time to completion when compared to the PLA results. Carter et al. (2004) hypothesised that mouth rinsing with a CHO solution can improve time trial perform- ance through the activation of oral receptors that can trigger the reward pathways of the brain and body.

The majority of studies that investigate the effects of CHO mouth rinsing on performance have focused on cycling performance, there have been studies which have investigated the effects of CHO mouth rinsing on other endurance sports (Dorling & Earnest, 2013; Kasper et al., 2016; Rollo, Cole, Miller, & Williams, 2010; Rollo, Williams, Gant, & Nute, 2008). Rollo et al. (2008) investigated the effects of CHO rinsing of running performance after an overnight fast, using similar administering and expelling techniques used in previous studies, giving a 25 ml bolus at predetermined stages in

Correspondence: Simon Devenney, Department of Science, Centre for Exercise and Metabolic Science, Institute of Technology Tallaght, Tallaght, Dublin, Ireland. E-mail: simon.devenney@ittd.ie

both the warm-up and time trial. The overall study results showed that CHO rinsing has a positive effect on running performance, with running speeds quicker in the first 5 min of the 30 min trial in the CHO group when compared with the PLA group. It was also indicated that the CHO group covered more distance in the first 5 min of the trial while also having a 1.7% increase in total distance covered when compared with the PLA group. Recent work by Kasper et al. (2016) investigated the effects of CHO rinsing and caffeine on high- intensity interval running capacity in a carbo- hydrate-restricted state. After completing a glycogen depletion test 24 h prior to the exercise session, par- ticipants completed a 45 min steady-state run (65% VO_2max) followed by a high intensity interval running (HIT) protocol consisting of 1 min at 80% followed by a minute at 60% until the onset of fatigue. The study showed that the use of CHO rinse and caffeine ingestion increased the exercise capacity of individuals during HIT when compared with a PLA and a standalone CHO rinse.

The majority of CHO rinse studies have investi- gated concentrations of 6% or 6.4%, with more recent studies looking at a higher concentration (Lane, Bird, Burke, & Hawley, 2012). A 1.8% improvement in time trial performance was noted when a 10% CHO solution was rinsed for 10 s with participants monitored in a fed state, with 3% improvements shown in a fasted state (Lane et al., 2012). These findings compare favourably to the pre- vious research findings of the 6% and 6.4% studies, showing an improvement in performance linked to activation of oral receptors. Not all the studies which have investigated CHO rinsing have discov- ered positive findings. Beelen et al. (2009) showed no enhancement in performance when mouth rinsing a CHO solution (6.4% maltodextrin) while in a fed state, while there was also no difference when comparing the CHO solutions to the PLA sol- ution. The results showed no significant difference in performance times in the CHO or PLA group, while also stating no difference was seen in the heart rate or power output.

It has been well documented to date that mouth rinsing of a 6% or 6.4% CHO solution can have a positive effect in relation to performance in a fasted state; however, the effect of this concentration in a fed state is unclear. The aim of the current study is to develop the foundations set by Beelen et al. (2009) and Lane et al. (2012) by investigating the effects of rinsing with the traditional CHO rinse sol- ution (6%) and a higher concentration (in this situ- ation a 16% CHO) have on exercise performance when compared with a 0% solution while participants are in a fed state. By using a 6% and 16% solution, the current study is able to compare two solutions which can replicate two commercially available sports drinks and determine their benefits and also to determine if there is a concentration dependence with regards to exercise performance and one could hypothesise that by comparing one concentration to another, performance improvement could be further enhanced with the use of a higher concen- tration of CHO due to a greater saturation of oral receptors.

Methods

Recruitment: Twelve recreationally active males vol- unteered to participate in this study (age 22 ± 7 years, body weight 69 ± 9 kg, height 1.75 ± 0.07 m, body-mass index 22 ± 1.7 kg/m^2, W_{max} 260 ± 28 W, VO_2max, 51 ± 3 ml kg^{-1} min^{-1}), all of whom had engaged in cycling activities (3–5 days a week/5–8 h per week). All participants were informed verbally and in written form of the study design and the physiological demands they would be placed under. Each individual completed a medical questionnaire and consent form prior to testing. The study was approved by the local research ethics committee.

Overall study design: The protocol consisted of four visits to the laboratory with all tests carried out on a cycle ergometer (Wattbike Pro, British Cycling Wattbike, Nottingham, England). Visit 1 involved a RAMP test to determine each participant's maximum aerobic power or work capacity (W_{max}). In the remaining three visits, individuals completed a set amount of work, which was individualised and calculated based on their maximum work capacity, in the shortest timeframe possible. In a randomised, repeated measures and double blinded study, partici- pants rinsed a 6% or 16% CHO solution or 0% PLA around the mouth at predetermined intervals.

Activity and diet before experiments: Participants were asked to keep a two-day training diary prior to each test visit, while they were allowed to undertake low-intensity exercise (heart rate below 150 beats/ min) for up to 2 h. Along with the training diary, a two-day dietary diary was recorded in order to prevent the disruption of the results. Participants were also asked to refrain from consuming alcohol and caffeine in the 24 h prior to each visit. The dietary diaries were a tool used to ensure participants consumed the same diet on the 2nd, 3rd and 4th visit.

Participants consumed a meal 2–3 h before testing (49 ± 2% carbohydrates, 18 ± 1% protein and 33 ± 2% fat) which was recommended by the authors.

Maximum workload capacity protocol: The maximum aerobic capacity test is a modified protocol which was based on the protocol performed by Beelen et al. (2009). Each participant performed an all-out incremental exercise test which was used to determine their W_{max}. Each participant underwent a 5 min warm-up at 100 W. On completion of the warm-up, the workload was set at 150 W and increased by 25 W every 2.5 min until the onset of exhaustion instead of the 50 W increase (Pottier, Bouckaert, Gilis, Roels, & Derave, 2010). The increased workload of each stage throughout this pro- tocol was influenced by an increase in cadence. Heart rate (Polar RS200, Polar Electro, Finland), rating of perceived exertion (RPE) and cadence were recorded on the completion of each interval. W_{max} of each indi- vidual was calculated using the following formula: $W_{out} + (t/150) \times 25$, where W_{out} is the watts of the last complete stage and t is the time spent in the final unfinished stage (Kuipers, Keizer, Brouns, & Saris, 1987).

Time-trial protocol: Prior to the commencement of the test protocol, each participant was weighed and baseline blood lactate levels were recorded and indi- viduals were fitted with a heart rate monitor (Polar RS200, Polar Electro, Finland) which was linked with the Wattbike. Participants endured a 5 min warm-up at approximately 40% of W_{max}, during which they were familiarised with the BORG scale of perceived exertion (RPE) (Borg, 1982, 1990). Fol- lowing completion of the warm-up, participants were asked to complete a set amount of work in the quick- est time possible. The total amount of work to be completed was calculated using a modified version of Jeukendrup, Saris, Brouns, and Kester (1996) equation:

$$\text{Total amount of work in Joules} = 0.65 \times W_{max} \times 3,600.$$

The equation by Jeukendrup et al. (1996) calcu- lated 75% of the participants W_{max} for endurance trained athletes, although due to recreational nature of the participants in the study and with evidence from previously unpublished work, the authors sought to modify the equation to 65%. The time trial protocol employed is designed to standardise workload so that each subject takes approximately 1 h to complete the work. The Wattbike was kept at a uniform resistance (resistance of 4) throughout the time trial performance for each participant in order to maintain a similar intensity for each partici- pant. Participants were only able to view the total amount of work they had performed, with heart rate, time and cadence values blocked from view and clocks removed to prevent participants knowing the time. No encouragement was offered throughout the test and the only interaction was when solutions were given for rinsing at 12.5% completed intervals, or to record HR, cadence and RPE at every 12.5% of completion. Laboratory conditions were held con- stant (ambient temperature 18–21°C) throughout each trial, with participants cooled using an electric fan.

Mouth rinse protocol: Over the three time trial visits, participants would use a 0% (PLA), 6% or 16% CHO solution (maltodextrin, due to lack of colour and taste). Each sample was a 25 ml bolus which was weighed before and after mouth rinsing, which was to ensure none of the sample was ingested. A bolus was provided to the participant after the warm-up and every 12.5% of completion in the trial. The sol- ution was rinsed around the mouth for 5 s before being expelled into a pre-weighed container. Sol- utions were made by an external researcher who was not affiliated to the study, to ensure the trial remained a double-blind study.

Statistical analysis: All collected data were ana- lysed using SPSS (Version 22.0, Chicago, IL). The variables were compared using a one-way repeated measure ANOVA, which was done to examine the effects across the three time trial per- formances and their corresponding solutions (0% PLA, CHO 6% and CHO 16%). Overall time of completion, average power output, cadence and speed were compared between each trial. Heart rate and RPE were compared at each individual stage across all three tests and were analysed using two-way repeated measures ANOVA. All data are represented using mean ± standard devi- ation with significance set at $p < .05$.

Results

Performance time and power output: In relation to time trial performance, both CHO solutions were signifi- cantly faster in comparison with the PLA trial, as per- formance times for the 6% CHO versus the PLA trial were 58.8 ± 7.0 min versus 62.3 ± 7.6 min ($p = .002$) while performance times for the 16% CHO trial versus the PLA trial were 57.9 ± 7.6 ($p = .001$). The individual differences in time to completion across all trials are shown in Figure 1(c).

A significant difference was also observed in average power output and average speed across the three trials. When comparing the 6% trial versus PLA and 16% trial versus PLA, power outputs were 174 ± 20 W versus 163 ± 23 W (p = .002) and 177 ± 23 W (p =.001), respectively. However similar to performance time, no significance was reported in power output when comparing the 6% trial to the 16% trial (p = .291). A non significant difference was observed in the average speed maintained when comparing the 6% and 16% trial (p = .273),

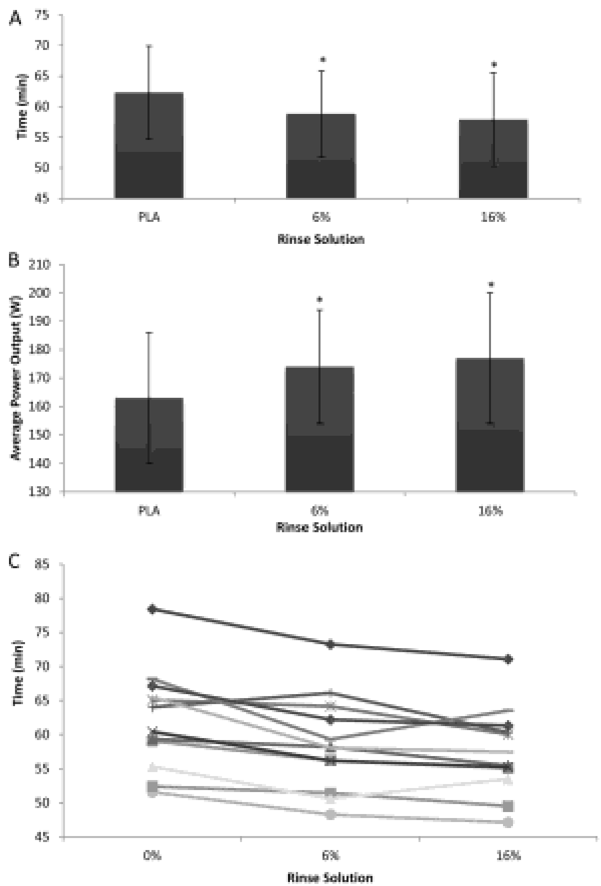

Figure 1. Performance times (A), average power output (B) and individual performance times (C) in the placebo and carbohydrate trials with values expressed as Mean ±SD. (A)* statistical difference p<0.05; (B)*statistical p<0.05.

significance was seen when either CHO trial, 6% or 16%, was compared with the PLA trial (34.8 ± 1.6 km h^{-1} versus 34.1 ± 1.7 km h^{-1}, p = .002, and 35.1 ± 1.8 km h^{-1}, p = .001). Power output and speed were typically observed to be higher in the first 10 min and final 15 min across in both carbo- hydrate trials in comparison to the PLA trial.

Heart rate and RPE: Values for heart rate and RPE were seen to increase steadily with the onset of exer- cise across the three trials. Average heart rate values of the PLA, 6% and 16% trials were 148 ± 18, 153 ± 20 and 153 ± 15 bpm, respectively, with maximal values for the PLA, 6% and 16% trials reaching 168 ± 18, 171 ± 19 and 174 ± 14 bpm, respectively. There were no differences in heart rate responses across the three trials (p > .005). Similar to heart rate, RPE values steadily increased throughout the three trials, with average values of 14.3 ± 1.07, 14.2 ± 1.7 and 13.8 ± 1.5, for the PLA, 6% and 16% trial, respectively. Maximum RPE values were recorded at 16.3 ± 1.5, 17.4 ± 1.7 and 17.3 ± 1.9, for the PLA, 6% and 16% trial, respectively. A non significant difference was reported in RPE values across the three trials (p > .005).

Rinse solution detection: From the 12 participants in the study, 4 were able to distinguish a difference in the mouth rinsing solutions used across the three time trials, reporting a difference in feel or viscosity of the solutions. Out of the four who reported a differ- ence, three performed better in the 16% trial when compared with both the 6% and PLA trial, while the fourth performed better in the 6% trial when compared with both the 16% and PLA trial. The other nine participants could not distinguish any difference across the three solutions.

Discussion

The aim of the current study was to investigate the effects mouth rinsing with a 6% and 16% CHO have on exercise performance in comparison to a 0% solution while participants are in a fed state. The current study shows that when compared to a PLA, the use of carbohydrate mouth rinse can lead to improvements in performance times, average power outputs and average speed during a time trial performed in a fed state. Early studies have investi- gated the effects of carbohydrate mouth rinsing on performance during high-intensity exercise. Carter et al. (2004) reported improvements of 2.9% in cycling time trial performance with use of a maltodex- trin mouth rinse, while improvements of 3.7% were reported when a mono and disaccharide sports rinse was substituted instead of the maltodextrin mouth rinse (Pottier et al., 2010). However, studies by Whitham and McKinney (2007) and Beelen et al. (2009) did not support these findings, reporting no difference in performance times when comparing a carbohydrate mouth rinse to a PLA. The studies by Carter et al. (2004), Pottier et al. (2010) and Whitham and McKinney (2007) all investigated the performance benefits of carbohydrate mouth rinsing within a fasted state, while the study by Beelen et al. (2009) looked at the benefits in a fed state. The current study took the same premise as the study by Beelen et al. (2009), with 12 participants performing three separate time trials while in a fed state, where they were provided with a 6% CHO, 16% CHO or PLA mouth rinse solution at every 12.5% of the trial completed. The use of nutritional diaries and same day/time testing enabled the moni- toring of each participants fed status. The results show improvements in time to completion in both CHO trials in comparison with the PLA trial, with significant improvements in time of completion in both CHO time trials. This improvement in time of completion is associated with each participant's ability to sustain both greater power output and higher speed during the 6% and 16% trial. Both heart rate responses and session RPE were similar across the three trials. By testing the participants of the current study in a fed state as opposed to a fasted state, the practical relevance of carbohydrate mouth rinsing can be determined.

It is believed that the CHO mouth rinse stimulates the reward regions of the brain via oral receptors due to the caloric content of the CHO (Chambers, Bridge, & Jones, 2009; Turner, Byblow, Stinear, & Gant, 2014). One would think that this stimulation of the reward pathways would lead to lower RPE levels observed in the CHO trials compared to the PLA. However, similar to previous reports no differ- ences were observed in RPE between either CHO trial and the PLA trial. The concentration and rinse duration may also have an impact on the effectiveness of the CHO rinse. The vast majority of the current research has use of a 6% or 6.4% CHO rinse solution for 5 s duration (Beaven, Maulder, Pooley, Kilduff, & Cook, 2013; Carter et al. 2004; Dorling & Earnest, 2013; Pottier et al., 2010; Rollo et al., 2008, 2010), while improvements in time trial performance have been noted with a greater solution concentration and longer rinse duration in both fed and fasted states (Lane et al., 2012). A greater concentrated sol- ution consisting of 16% maltodextrin was compared to the traditional 6% solution and PLA in the current study. As previously stated both CHO rinse solutions improved cycling performance in comparison to a PLA (improvements of 5.6% and 7.1% observed), although no significant difference was observed when comparing both CHO trials to each other. Using a higher concentration of mouth rinse may enhance the saturation of the oral receptors leading to a greater stimulation of the reward path- ways reported by Chambers et al. (2009) and Turner et al. (2014), although further investigation on the mechanism is required.

Conclusion

The applications of carbohydrate rinsing have grown since the first study by Carter et al. (2004), with the current literature investigating the effects in sports ranging from cycling (Beelen et al., 2009; Fares & Kayser, 2011; Lane et al., 2012; Pottier et al., 2010) to running (Rollo et al., 2008, 2010; Rollo & Williams, 2011; Whitham & McKinney, 2007), with more recent studies inves- tigating the effects on field sport simulation and strength work (Dorling & Earnest, 2013; Jensen, Stellingwerff, & Klimstra, 2015; Painelli et al., 2011). With the ergogenic benefits well documented, the data can translate from the scien- tific field to the practical setting for athletes and coaches alike, as the current study along with other findings suggest that CHO rinsing at regular intervals may benefit those athletes reporting gas- trointestinal problems. In conclusion, the present study shows that the use of carbohydrate mouth rinsing during high-intensity exercise can lead to an improvement in exercise capacity during a simu- lated time trial when exercise is carried out in a fed state.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Appendix 2: Devenney, S., Mangan, S., Shortall, M., & Collins, K. (2018). Effects of carbohydrate mouth rinse and caffeine on high-intensity interval running in a fed state. Applied Physiology, Nutrition, and Metabolism, 43(5), 517-521.

ARTICLE
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Effects of carbohydrate mouth rinse and caffeine on high-intensity interval running in a fed state

Simon Devenney, Shane Mangan, Marcus Shortall, and Kieran Collins

Abstract: The current study aims to identify if mouth rinsing with a 6% carbohydrate mouth-rinse (CMR) solution and mouth rinsing and ingestion of caffeine (CMR+CAFF) can affect exercise performance during steady-state (SS) running and high- intensity intervals (HIIT) in comparison with a 0% control solution (PLA) when in a fed state. Eight recreationally trained males completed 3 trials (CMR, CMR+CAFF, and PLA) of 45 min SS running and an HIIT protocol (90% peak treadmill velocity) until fatigue in a double blinded, repeated-measures study. Participants ingested a capsule of either CAFF or PLA before and after SS. Participants received a 25-mL bolus of carbohydrate solution (CMR and CMR+CAFF trials) or taste-matched PLA (PLA trial) prior to HIIT protocol and after every second effort. Heart rate and lactate responses were recorded throughout the SS and HIIT protocol. CMR+CAFF was significantly different when compared with PLA (p = 0.001; Cohens d = 1.34) and CMR (p = 0.031; Cohens d = 0.87) in relation to distance covered before fatigue. Although there was no significant difference between CMR and PLA, there was a small benefit for CMR (p = 0.218; Cohens d = 0.46). Results indicate that CMR and ingestion of CAFF leads to improvements in performance during interval sessions while participants were in a fed state. These findings indicate that the regular use of CMR can decrease the risk of gastrointestinal distress reported by athletes, which can be applicable to athletes in a real-world setting.

Key words: mouth rinse, carbohydrate, caffeine, high intensity, interval running.

Résumé : Dans cette étude, on vérifie si l'utilisation d'un rince-bouche contenant 6 % de sucre (« CMR ») et l'utilisation d'un rince-bouche combinée à la consommation de caféine (« CMR+CAFF ») ont un effet sur la performance au cours d'une séance de course en régime stable (SS) et par intervalles d'intensité élevée (« HIIT ») comparativement à une solution de contrôle contenant 0 % d'ingrédient (« PLA ») sans être à jeun. Huit hommes actifs par loisir participent, selon un devis à double insu avec mesures répétées, à trois essais (CMR, CMR+CAFF et PLA) comportant 45 min de course SS suivie d'un HIIT (90 % de la vitesse de pointe sur tapis roulant) jusqu'à épuisement. Les participants consomment une capsule du placebo ou de caféine avant et après SS. Les participants consomment 25 mL de solution sucrée (essais CMR et CMR+CAFF) ou d'un placebo ayant le même goût (essai PLA) avant le protocole HIIT et après chaque deuxième effort. On enregistre les valeurs de la fréquence cardiaque et de la concentra- tion de lactate tout au long de SS et du protocole HIIT. La condition CMR+CAFF révèle des résultats significativement différents comparativement aux conditions PLA (p = 0,001; d de Cohen = 1,34) et CMR (p = 0,031; d de Cohen = 0,87) en ce qui concerne la distance franchie avant l'épuisement. Bien qu'il n'y ait pas de différence significative entre CMR et PLA, la condition CMR présente un léger bénéfice (p = 0,218; d de Cohen = 0,46). D'après les résultats, la condition CMR+CAFF suscite une amélioration de la performance au cours des intervalles de course chez les participants non à jeun. D'après les observations, l'utilisation régulière d'un CMR peut diminuer le risque de malaises gastro-intestinaux rapportés par les athlètes; ces observations pour- raient s'appliquer aux athlètes en milieu naturel. [Traduit par la Rédaction]

Mots-clés : rince-bouche, sucre, caféine, intensité élevée, course par intervalle.

Introduction

Early carbohydrate mouth-rinse (CMR) studies reported improvements in both running and cycling time trial performance (Carter et al. 2004; Pottier et al. 2010; Rollo and Williams 2011; Rollo et al. 2008). Carter et al. (2004) investigated the effects of a 6.4% maltodextrin solution on a 1-h time trial in comparison with a 0% solution. Improvements of 2.9% were reported for the aver- age time to completion and the authors hypothesised that CMR's improvements in time-trial performance is linked to the activa- tion of the neural pathways via the oral receptors (Chambers et al. 2009).

Whilst CMR studies have primarily been focused on cycling performance (Beelen et al. 2009; Carter et al. 2004; Chong et al.

2011; Lesniak et al. 2016), studies have shifted some focus to inves- tigating the effects of CMR on other endurance sports (Dorling and Earnest 2013; Kasper et al. 2016; Rollo et al. 2008, 2010). Fol- lowing an overnight fast, Rollo et al. (2008) investigated the effects of CMR on running performance with results showing a positive effect in relation to running speeds and total distance covered due to the CMR. Not all the findings from studies investigating CMR have been positive, as work by Beelen et al. (2009) showed no enhancement in power output, heart rate, or time to completion with a CMR (6.4% maltodextrin) whilst individuals were in a fed state. More recent work by Devenney et al (2016) demonstrated that when in a fed state, a 6% CMR (maltodextrin) can elicit a 5.6% performance improvement in cycling performance. With the ef- fects of 6% and 6.4% solutions well documented, more recent stud-

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S. Devenney, S. Mangan, M. Shortall, and K. Collins. Department of Science, Centre for Exercise and Metabolic Science, Institute of Technology Tallaght, Dublin, Ireland.

Corresponding author: Simon Devenney (email: Simon.Devenney@it-tallaght.ie).

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ies have investigated higher concentrations CMR (Devenney et al. 2016; Lane et al. 2013). Improvements of 1.8% were reported in time-trial performance with a 10% solution in a fed state, while improvements of 3% were reported in a fasted state (Lane et al. 2013), while Devenney et al. (2016) reported improvements of up to 6.1% with a 16% solution when compared with a 0% placebo (PLA). These findings compare favourably to previous studies of 6% and 6.4% solutions, showing increases in exercise performance linked to oral receptor activation (Chambers et al. 2009; Frank et al. 2008). These authors found that the use of CMR increased activation of the reward region of the brain, mainly the ventral striatum and anterior cingulate cortex (Chambers et al. 2009; Frank et al. 2008).

Caffeine (CAFF) ingestion can enhance repeated sprint performance in both running (Carr et al. 2008; Mohr et al. 2011) and cycling (Lee et al. 2011). Kalmar (2005) reported that this endurance and sprint performance enhancement may be due to a mechanism of motor unit activity along with adenosine receptor antagonism. Recent work by Kasper et al. (2016) investigated the effects of CMR and the supplementation of CAFF on anaerobic running capacity in a glycogen depleted state. Participants completed a 45-min steady-state (SS) (65% maximal oxygen uptake ($\dot{V}O_{2max}$)) run followed by an HIIT exercise capacity (1 min at 80% $\dot{V}O_{2max}$ interspersed by 1 min at 60% $\dot{V}O_{2max}$) until fatigue. The findings demonstrated an increased exercise capacity with CMR and CAFF ingestion (CMR+CAFF) when compared with a PLA and CMR by itself. Other work by Kizzi et al. (2016) investigated the effects of a CAFF rinse compared with both a control and PLA while participants were in a low endogenous carbohydrate state. Although the study showed that peak power output was greater in the control, CAFF rinse prevented a drop-off in power outputs across a repeated-cycling sprint protocol in comparison with both the control and PLA. Whilst the majority of the previously mentioned research has been carried out when athletes are in a fasted state, there is a greater need for research findings demonstrating the benefits of CMR in a fed state, as it is rare that athletes will endure competition in a fasted state. Although recent work by Lane et al. (2013) and Devenney et al. (2016) has begun to show the benefits of CMR in a fed state, more detailed work in the area is needed. The aim of this study was to ascertain whether CMR, either alone or in combination with CAFF, could enhance the high-intensity running performance of recreationally trained athletes in a fed state, when compared with a PLA.

Materials and methods

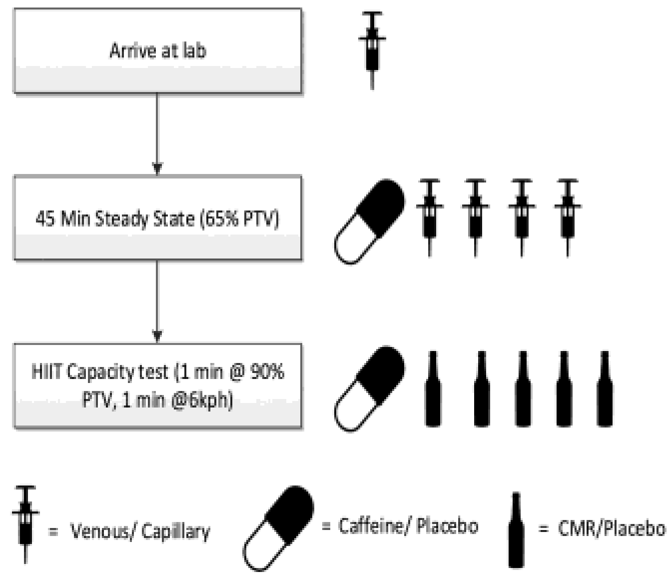
Recruitment

Eight recreationally trained males volunteered to participate in this study (age: 23 ± 3 years; body mass: 78 ± 7 kg; height: 1.75 ± 0.05 m; body mass index: 22 ± 1.7 kg·m^{-2}; $\dot{V}O_{2max}$: 51 ± 3 mL·kg^{-1}·min^{-1}), all of whom had engaged in exercise of 5–8 h per week. All participants were informed of the study both verbally and in written form along with an outline of the physiological demands for each testing session. Medical questionnaire and consent forms were completed for each participant prior to testing. The study was approved by the Ethics Committee of Institute of Technology Tallaght.

Overall study design

In a randomised, repeated-measures and double-blinded study participants undertook an SS run (45 min at 65% peak treadmill velocity (PTV)) followed by an HIIT protocol until exhaustion (1-min bouts at 90% PTV, separated by a 1-min bout of walking at 6 km·h^{-1}). Participants consumed a standardised CAFF capsule (200 mg) or capsulated PLA prior to the SS running and a second CAFF or PLA capsule was consumed prior to the commencement of the HIIT protocol. Throughout the HIIT protocol participants rinsed with a 6% carbohydrate (maltodextrin) solution for 5 s on completion of every second interval. Therefore, each participant undertook 3 experimental trials that consisted of PLA capsule and PLA mouth rinse (PLA trial), PLA capsule and CMR (CMR trial), and CAFF capsule and CMR (CMR+CAFF trial). The primary marker during this study was time to fatigue in the HIIT protocol. Heart rate (HR), blood lactate, and rate of perceived exertion were monitored throughout the SS run, while HR responses were monitored throughout the HIIT protocol. Figure 1 gives an overview of the experimental design.

Fig. 1. Schematic overview of the testing session. CMR, carbohydrate mouth rinse; HIIT, high-intensity interval training; PTV, peak treadmill velocity.



Assessment of $\dot{V}O_{2max}$

A week prior to their first trial, each participant completed a graded exercise test on a motorised treadmill (Cosmed T170, Roma, Italy) to determine $\dot{V}O_{2max}$. The protocol used was based on that of Akubat et al. (2014), with 4-min stages interspersed with 1-min recovery. Stages started at 8 km/h with increments of 2 km/h after completion of each stage (8, 10, 12, 14, 16 km·h^{-1}) until onset of blood lactate accumulation, which was followed by a ramp protocol (increments of 1 km·h^{-1}·min^{-1}) to volatile exhaustion. Breath by breath analysis was recorded throughout the test using the Cosmed Quark CPET Metabolic Cart, Gas Analyser (Cosmed, Roma, Italy) with achievement of $\dot{V}O_{2max}$ determined by plateau of oxygen consumption despite an increase of workload and a respiratory exchange ratio value greater than 1.1. Running speeds for the experimental trials were determined from PTV. Following graded exercise tests, participants undertook a familiarisation process for the speeds at both the SS (65% PTV) and HIIT (90% PTV), so participants could familiarize themselves to the demands required in the remaining 3 visits.

SS exercise protocol and HIIT protocol

Kasper et al. (2016) implemented a 45-min SS and chose to administer CAFF prior to the SS based on findings from Graham and Spriet (1995), which demonstrated that peak plasma CAFF typically occur 45-min after ingestion. Therefore, this study implemented a similar 45-min SS set at 65% PTV for each participant. Participants arrived at the laboratory at their allocated time and were given either 200 mg of CAFF (Bulk Powders, Essex, UK) or a visually identical PLA (Whey Protein Isolate; Bulk Powders) prior to, and immediately after, the completion of the SS exercise protocol. The second CAFF capsule was administered prior to the HIIT protocol as a means of further developing the ergogenic effect. HR, rating of perceived exertion (RPE) (Borg 1982; Borg et al. 1987), and blood lactate were monitored throughout the SS exercise at time points of 5, 10, 20, 30, 40, and 45 min. Following the SS protocol, participants commenced an HIIT protocol that consisted of 1-min bouts at 90% PTV followed by a 1-min bout of walking at 6 km·h^{-1} until physical exhaustion. Time to fatigue was calculated at the time which the participant could no longer meet the demands of the protocol. This follows similar methodology of prescribing interval where specific velocities are used to prescribe intervals (Buchheit and Laursen 2013) Participants rinsed a 25-mL bolus containing either a 6% carbohydrate beverage (Maltodextrin; Bulk Powders) or a taste-matched (orange) and visually identical PLA (Robinson Squash; Britvic Orange Soft Drinks, Hertsfordshire, UK) for 5-s periods every 4 min during exercise before expectorating.

Activity and diet before experiments

A 2-day training diary was kept by each participant prior to each test visit, with low-intensity exercise (HR below 150 beats·min^{-1}) permitted for up to 60 min the day prior to testing. Alcohol and CAFF consumption was not permitted in the 24 h prior to each visit. Dietary intake was recorded and was replicated for each visit as a measure to prevent the disruption of the results. Participants consumed a meal 2–3 h before testing (49% ± 2% carbohydrates, 18% ± 1% protein, and 33% ± 2% fat) with each meal recommended to the participants by the authors.

Statistics

Data collected were analysed using a 1-way repeated-measures ANOVA (IBM SPSS, version 24 for Windows; IBM Corp.). Before running the required ANOVA to investigate differences in exercise capacity (distance running during HIIT protocol) we performed a Shapiro–Wilks test to assess the data for normality. In the event that any statistically significant differences arose, a post hoc analysis was conducted comprising a paired *t* test with a Bonferroni adjustment of the level of alpha for multiple comparisons. Values are expressed as the means ± SD with significance set at $p < 0.05$. Effect sizes (ESs) and 95% confidence intervals (95% CIs) are reported for applicability to the general population. ESs are reported in line with previous recommendations (Cohen 1992; Lakens 2013), using thresholds of 0.2 (small), 0.6 (moderate), and 1.2 (large) (Hopkins et al. 2009) and effects of 0.19 or less deemed trivial.

Results

Distance to fatigue

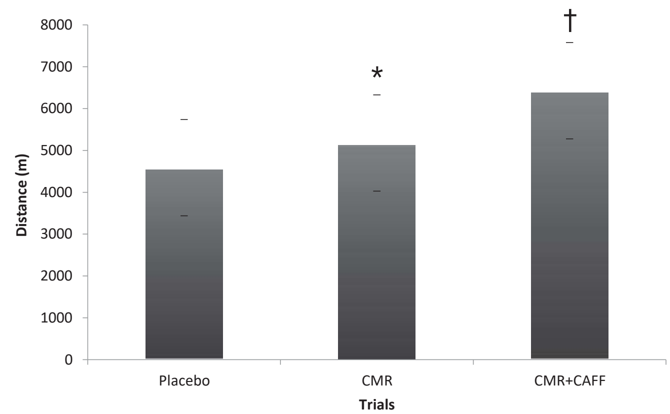
In relation to running performance there was a significant increase in distance covered between the PLA and the CMR+CAFF (p = 0.001, 95% CI: 1036–2645 m; Cohen's d = 1.34) and between CMR and CMR+CAFF (p = 0.031, 95% CI: 131–2367 m; Cohen's d = 0.87). However, there was no significant difference reported between the PLA and CMR (p = 0.218, 95% CI: –302 to 1484 m; Cohen's d = 0.46), although ES can indicate a possible benefit. Mean distance covered across the individual trials were as follows: PLA, 4535 ± 1217 (95% CI: 3634–5438) m; CMR, 5127 ± 1367 (95% CI: 4114–6140) m; CMR+CAFF, 6376 ± 1508 (95% CI: 5259–7493) m and are illustrated in Fig. 2.

Physiological responses

Values for HR were seen to increase steadily with the onset of exercise across the 3 trials with Table 1 illustrating HR responses at set time points. There was no significant difference in heart rate responses across the 3 trials ($p > 0.05$).

Similarly to HR, lactate responses remained constant throughout the SS running, while an increase was seen post-HIIT across all 3 trials. No significance was observed when comparing lactate responses between the experimental trials ($p > 0.05$). Again similarly to HR and blood lactate, no significance was reported in RPE scores across all trials ($p > 0.05$).

Fig. 2. Mean distance to fatigue during the HIIT exercise test across all experimental trials. *, A possible benefit compared with the PLA; †, a significant benefit compared with the PLA. CAFF, caffeine ingestion; CMR, carbohydrate mouth rinse; HIIT, high-intensity interval training; PLA, placebo.



Rinse solution detection

None of the participants involved in the study were able to differentiate between the mouth rinsing solutions used across the 3 experimental trials. Each participant was unable to distinguish any difference in taste or viscosity of the solutions.

Discussion

The aim of the study was to ascertain whether CMR, either alone or in addition to CAFF supplementation, could augment high-intensity running performance in comparison with a PLA in recreationally trained males in a fed state. The findings from the study support the initial hypothesis of incremental benefit to running performance, similar to that of previous work by Kasper et al. (2016). Although the statistical certainty around the improvements in the CMR group are not as strong (p = 0.218) in relation to distance to fatigue, the CMR does illicit a small to moderate effect (Cohen's d = 0.46). We also confirm that CAFF maintains its ergogenic effects despite exercising in a fed state.

Previous work examining the effect of CMR in a postprandial state has been somewhat equivocal. Early work revealed no significant effect of CMR on exercise performance in a postprandial state (Beelen et al. 2009; Whitham and McKinney 2007) while more recent work has shown performance improvements of 1.8%– 7.1% (Devenney et al. 2016; Lane et al. 2013) albeit with higher solution concentrations in conjunction to the more traditional CMR concentrations. While the CMR did not produce a statistically significant benefit, the different CIs and moderate Cohen's d ESs would suggest that there is still a benefit elicited from the CMR condition. This would support previous work that has demonstrated that while there seems to be greater benefit in a fasted state, a benefit is still obtained from CMR even in the fed state (Devenney et al. 2016; Fares and Kayser 2011; Lane et al. 2013).

In an bid to further enhance exercise performance, CAFF capsules were consumed both pre- and post-SS sessions to allow peak plasma levels before the commencement of the HIIT protocol (Goldstein et al. 2010). Compared with a PLA, this addition of CAFF had a profound effect on running distance with significant difference in distance to fatigue and a large ES (Cohen's d = 1.34). This would indicate strong merit in the prescription of CMR+CAFF for those undertaking high-intensity running. Furthermore, not only did the CMR+CAFF condition have a significant benefit over the PLA condition, but a significant increase in running distance when compared with the CMR-only condition was also observed. Thus it seems the addition of CAFF exerts a moderate effect (Cohen's d = 0.87) on high-intensity running performance. This

Table 1. Mean HR, blood lactate, and RPE values across the 3 experimental trials.

	Time (min)						Exhaustion
	5	10	20	30	40	45	(min)
Placebo							
HR (beats·min^{-1})	155±15	162±14	167±17	171±13	171±15	172±15	188±12
Lactate (mmol·L^{-1})	2.2±0.9	2.3±1.0	2.5±1.1	2.5±1.2	2.5±1.1	2.4±1.2	9.8±2.3
RPE (Borg scale)	10±2	11±3	12±2	14±3	14±2	14±2	20±1
CMR							
HR (beats·min^{-1})	151±10	160±11	164±10	169±12	169±12	170±13	188±10
Lactate (mmol·L^{-1})	1.9±0.6	2.1±0.9	2.1±0.9	2.1±1.1	2.1±1.1	2.2±1.1	9.5±3.0
RPE (Borg scale)	9±2	9±3	11±2	13±2	14±2	13±3	20±1
CMR+CAFF							
HR (beats·min^{-1})	150±12	160±17	168±16	171±17	173±16	174±16	192±10
Lactate (mmol·L^{-1})	1.9±0.9	2.0±0.9	2.4±1.1	2.5±1.0	2.6±1.0	2.3±1.1	10.1±2.4
RPE (Borg scale)	9±2	10±2	11±1	12±3	13±3	13±2	20±1

Note: CAFF, caffeine ingestion; CMR, carbohydrate mouth-rinse; HR, heart rate; RPE, rating of perceived exhaustion.

study, alongside the work of Kasper et al. (2016), provides compelling evidence for the addition of CAFF to CMR to further augment performance. CMR is believed to stimulate the reward pathways of the brain via oral receptors owing to a carbohydrate solutions caloric content (Chambers et al. 2009; Turner et al. 2014). One would envision that this stimulation would illicit lower RPE levels in the CMR trials compared with the PLA trial, leading to a viable reason for the increase in exercise performance, as exercise intensity or tolerance can increase to compensate for the drop in RPE levels. However, previous research has reported a lack of a difference in RPE and HR responses (Beaven et al. 2013; Carter et al. 2004; Chambers et al. 2009; Devenney et al. 2016; Pottier et al. 2010) and the current study confirms the findings, with no significant difference reported in RPE or HR levels between either of the CMR or CMR+CAFF trials and the PLA trial.

There were limitations to consider within the current study. As participants were recreationally trained athletes, it is unknown if the current results can translate to the well-trained athletic population. An additional limitation of this study is the sample size (n = 8); therefore, the authors utilised ES to understand the prac- tical significance of the results. Although practical significance was found, the authors issue caution owing to the small sample size. Although subjects were reported to be low consumers of CAFF, CAFF habituation was not measured and therefore may have affected acute responses to the CAFF dose. There was no utilization of a non-rinse control, nor was the possibility of a dose–response addressed.

Conclusion

In conclusion, the study provides novel data that illustrates that CMR, whether alone or in tangent with caffeine supplementation, can delay the onset of fatigue in anaerobic interval running. With the benefits already reported, these findings along with previous research suggest that the regular use of CMR can decrease the risk of gastrointestinal distress reported by athletes, meaning the data can be applicable to both athletes and coaches in a real-world setting.

Conflict of interest statement

The authors report no conflicts of interest associated with this manuscript.

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Appendix 3: Devenney, S., Shovlin, A., Mangan, S., Malone, S., Shortall, M., & Collins, K. Effects of carbohydrate and caffeine mouth rinse on repeated sprint performance in a fed state.

Effects of carbohydrate mouth rinse, caffeine mouth rinse or a combination on repeat cycling sprints.

Simon Devenney,[1] *Aidan Shovlin,*[1] *Shane Mangan,*[1] *Shane Malone,*[2] *Kieran Collins,*[1] *Marcus Shortall.*[1]

1. Centre for Exercise and Metabolism Science, Institute of Technology Tallaght, Dublin.
2. Research Institute for Sport and Exercise Sciences, Liverpool John Moores, Liverpool, UK.

Previous research has shown that mouth rinsing with a carbohydrate solution can improve cycling performance while caffeine mouth rinse can improve power output in bouts of repeated sprints in a low carbohydrate availability state. The aim of the current study was to identify the effects of mouth rinsing with a 6% CHO solution, a 1.2% CAFF solution or combination (CHO/CAFF) on exercise performance during repeated sprint cycling intervals when compared to a 0% solution (PLA) and control, each in a fed state. Fourteen recreationally active males (aged 22 ± 3yrs, body mass 71.5 ± 10.1kg, stature 173.5 ± 7.6cm, skinfolds 75.6 ± 19.3mm, maximal power output [W_{max}] 305 ± 30W, $\dot{V}O_{2max}$ 52.7 ± 5.8ml·kg^{-1}·min^{-1}) completed five trials consisting of a six 10second sprints interspersed with fifty seconds active recovery. Participants received a 25ml bolus consisting of one of the four rinse solutions (PLA, CHO, CAFF or CHO/CAFF) before each interval rep. Heart rate and RPE responses were recorded at the end of each interval while lactate responses were recorded pre and post sprints. Results will be analysed using a Two-Way repeated measures Anova (SPSS 23.0) with $p<0.05$, while effect size are reported in order to infer applicability to the general population as well as certainty of outcomes respectively. Results indicate that in relation to peak power output of sprint 1, there is a small effect when using the CHO, CAFF or CHO/CAFF (Cohen's *d*, 0.40 to 0.53; *small*) versus the PLA and CONT. Similarly for the final sprint, there is a small effect when using the CHO, CAFF or CHO/CAFF rinse (Cohen's *d*, 0.23 to 0.28; *small*), in comparison to the PLA and CONT. No variation was observed when comparing the CHO to either the CAFF or CHO/CAFF. No difference was observed between HR levels or RPE values across all experimental trials with $p>0.05$. CHO and/or CAFF mouth rinses may rapidly enhance power production, which could benefit short sprint exercise performance. The ability for mouth-rinse to improve maximal exercise performance in the absence of fatigue further suggests a central mechanism.

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