THE EFFECTS OF CARBOHYDRATE MOUTH RINSE CONCENTRATION ON CYCLING TIME TRIAL PERFORMANCE

by

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ABSTRACT

Mouth rinsing with a carbohydrate (CHO) solution during exercise has been shown to improve endurance exercise performance. However, it is unclear if performance is improved to a greater extent with a higher concentration mouth rinse. **PURPOSE:** The purpose of this study was to determine if there was a dose-response effect to CHO mouth rinse concentration on endurance performance during a 1h cycling time trial. **METHODS:** Fourteen male participants, aged 18-45 years old, who cycled a minimum of 30 miles per week, participated in this study. Participants completed five, 1h time trials on a cycle ergometer, each separated by at least five days. During the first trial, participants completed a familiarization trial during which they rinsed with 25ml of water every 15 minutes of the time trial. In a double-blind fashion, participants then completed trials during which a 0%, 3%, 6%, or 12% CHO solution was rinsed in 15-minute intervals during the four experimental trials. Average power, work completed, heart rate (HR), rating of perceived exertion (RPE), and cadence were recorded during each trial. **RESULTS:** The results indicated that there were no significant differences in work performed ($p = 0.405$), average power ($p = 0.082$), HR ($p = 0.399$), or RPE ($p = 0.764$) across any of the experimental trials. **CONCLUSION:** This study found no improvement in cycling time trial performance when using a CHO mouth rinse and no dose-response to CHO mouth rinse concentration. Further research is warranted to investigate the possibility of a dose-response in a fasted state.

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CHAPTER ONE: INTRODUCTION

It has been well established that carbohydrates (CHO) play a vital role during exercise. Supplementing with CHO improves performance through a variety of mechanisms (Karelis et al., 2010). Although the entire picture is unclear, there are several pathways through which CHO work to improve performance (Karelis et al., 2010). These include: maintenance of blood glucose and prevention of hypoglycemia, improved cognition, maintenance of high CHO oxidation rates, possibly reducing the rate of glycogen depletion, lower cortisol, IL-6, and C-reactive protein, maintenance and protection of neuromuscular membrane excitability, improved handling of Ca^{2+} in the sarcoplasmic reticulum, and maintenance of the neural drive (Karelis et al., 2010). The American College of Sports Medicine Position Stand (Sawka et al., 2007) recommends consuming a 6-8% CHO solution that provides 30-60g•h^{-1} of CHO during high-intensity exercise lasting one or more hours. This amount of CHO has been shown to maintain plasma glucose levels, preserve liver glycogen, and sustain exercise performance (Sawka et al., 2007).

In 2013, nearly 12 million runners participated in over 20,000 races nationwide over distances ranging from 5ks to half marathons (Running USA, 2014). This underpins the popularity of these events in the United States. On the elite level, 10ks and half marathons typically last around 26-30 and 60-65 minutes, respectively. Prize money at these events continues to increase and competition by athletes from around the world is becoming more prevalent. Thus, maximizing performance on race day is extremely

important to these athletes. One of the problems many athletes face in these competitions is gastrointestinal (GI) discomfort caused by the consumption of food and/or liquids (van Nieuwenhoven et al., 2005). If there is an ergogenic effect from consuming CHO during competition, these athletes will not be able to benefit due to GI distress. van Nieuwenhoven et al. (2005) demonstrated that CHO beverage ingestion may even prove detrimental to running performance because of the increased incidence of GI discomfort.

Investigations into the ergogenic benefits of CHO supplementation during shorter duration endurance exercise (where glycogen depletion is not a limiting factor) have demonstrated improved performance when CHO are ingested. Subjects drinking a 7% CHO drink were able to achieve higher peak and mean power, and a reduced rate of fatigue in a Wingate Anaerobic Power Test following 50 minutes of high intensity cycling (Ball et al., 1995). For events such as a one-hour cycling criterium race, this is extremely beneficial, as the athlete must ride at a very high rate before performing a maximal effort sprint at the finish of the race. Jeukendrup et al. (1997) examined the effect of CHO feedings during a one-hour cycling time trial. Participants were able to complete a set amount of work in a shorter amount of time and maintain a higher power output when ingesting a CHO beverage compared with a placebo (Jeukendrup et al., 1997). Due to the low amount of exogenous CHO being oxidized during short distance exercise, it was hypothesized that some other mechanism must play a major role during exercise of one-hour or less.

Based on Jeukendrup's work and observations that intravenous infusion of glucose does not improve performance in 1h high-intensity cycling (Carter et al., 2004b), Carter el al. (2004a) were the first to investigate whether CHO exert an ergogenic effect

on central motor control through oral receptors. Cyclists completed a set amount of work that was selected to last approximately 1h while rinsing with either 25mL of water or a 6.4% maltodextrin solution periodically throughout the time trial. Power output was higher and time to completion was lower in the CHO mouth rinse trial even while heart rate and RPE remained the same between trials (Carter et al., 2004a).

Since 2004, over 20 studies have investigated the benefits of mouth rinsing with CHO (Burke & Maughan, 2015). Studies on recreational and trained athletes have examined a number of different protocols, all of which last approximately one-hour. The results of a majority of these studies indicate that mouth rinsing with CHO is beneficial and activates centers of the brain responsible for motor control (Chambers et al., 2009). Mouth rinse protocols vary widely among studies and limited evidence exists related to the potential dose-response of greater concentrations of CHO in the mouth rinse. Wright and Davison (2013) found no difference in performance between a 6% and 12% CHO mouth rinse during a 1.5h run. Bottoms et al. (2014) showed no difference in performance during a 30min cycling time trial when rinsing with a 3%, 6%, or 12% solution. This duration may be too short to see any differences. As well, Ispoglou et al. (2015) showed no cycling time trial performance differences when rinsing with a 4%, 6%, and 8% CHO solution.

Need of the Study

Research has established that CHO mouth rinses are beneficial during endurance exercise and are an easily administered method to enhance performance (Burke & Maughan, 2015; De Ataide e Silva et al., 2014). To date, three studies (Bottoms et al., 2014; Ispoglou et al., 2015; Wright & Davison, 2013) have investigated whether there is

an improvement in performance with higher CHO concentration mouth rinses. The varied exercise protocols, specifically the 30min protocol of Bottoms et al. (2014) which may be too short of a duration and the small sample sizes of these studies limit the conclusions that can be drawn regarding a dose-response effect to CHO mouth rinse concentration and warrants the need for more research on the topic.

Purpose

The purpose of this study was to determine if there is a dose-response effect to different concentrations of CHO mouth rinse on 1h cycling time trial performance. Specifically, this study investigated the exercise response to mouth rinsing a 0, 3, 6, and 12% CHO beverage.

Hypothesis

A greater amount of work will be accomplished during a cycling time trial in which participants rinse with a 12% CHO solution followed by rinsing with a 6, 3, and 0% CHO solution respectively.

Significance of the Study

This study will help to determine if a dose-response effect to CHO mouth rinsing exists and leads to improved performance for endurance activities lasting around an hour in duration. It will also support previous literature suggesting that mouth rinses work through oral sensation of nutrient density rather than beverage sweetness (Gant et al., 2010). There are a number of athletes who can use the mouth rinse protocol for races from a 10k run to a 40km cycling time trial to improve performance while limiting GI distress due to fluid ingestion.

CHAPTER TWO: LITERATURE REVIEW

Sports drinks are a mainstay during competitive sports ranging from cycling and running, to American football, soccer, and basketball. Recently, it has been proposed that CHO in sports drinks exert some of their ergogenic effect without actual ingestion of the beverage (Carter et al., 2004a). This review of literature will discuss the benefits of CHO during endurance exercise studies, with specific focus on CHO mouth rinses, their mechanism of action, and the effects of mouth rinse concentration and prandial state on performance.

CHO Use During Endurance Exercise

The consumption of CHO during endurance exercise serves a number of purposes that ultimately contribute to maintaining high power outputs and increasing athletic performance. This is demonstrated in events ranging from half marathons to multi week events such as the Tour de France where up to 30% of riders' daily CHO intake can come solely from CHO beverages consumed during the races (Saris et al., 1989).

Energy Production During Endurance Exercise

Of the three macronutrients (CHO, fats, and protein), CHO and fat are the primary sources of energy during exercise (McArdle, Katch, & Katch, 2013). Intensity will determine the relative ratio of CHO to fats oxidized during the activity. As intensity increases from rest to maximal effort, the body shifts from predominantly utilizing fats to almost exclusively using CHO, termed the "crossover" effect by Brooks and Mercier (1994). As much of an athletes training is done above 70% $\dot{V}O_{2\,max}$, CHO from muscle

and liver glycogen and blood glucose are the preferred fuel sources for such intensity of exercise. It is not uncommon for competitions to elicit a $\dot{V}O_2$ greater than 90% $\dot{V}O_{2\,max}$, which would be even more reliant on CHO as a fuel source.

Whereas CHO oxidation increases with increasing intensity, the opposite is true when considering exercise duration. Fat oxidation increases as exercise continues for several hours as glycogen breakdown is slowed. Liver glycogen continues to maintain blood glucose levels until its own stores are depleted and the rate of muscle uptake exceeds that of the liver's production (Noakes, 2004). If exercise intensity is maintained at a high level (70-74% $\dot{V}O_{2\,max}$) without CHO supplementation, fat oxidation increases until a point when the athlete's cardiorespiratory system is no longer able to supply the muscle mitochondria with enough oxygen to maintain a high enough level of fat oxidation to sustain the exercise intensity (Coyle et al., 1986).

Even though CHO play a vital role in exercise energy production, the human body has limited stores. An 80kg man has approximately 400g stored in muscle glycogen, 100g stored in liver glycogen, and 3g as circulating blood glucose, which combined provides 1500-2000kcal of energy (McArdle, Katch, & Katch, 2013). Thus, a 70kg runner competing in a 42.195km marathon at 1kcal•kg^{-1}•km^{-1} would exhaust all CHO stores well before the finish even with energy contributions from fat stores. Average energy expenditure during a stage of the 1989 Tour de France was estimated to be 6071 ± 335kcal•day^{-1} and peak energy expenditure was 7815 ± 382kcal•day^{-1} (Saris, 1989). CHO consumption during the daily races amounted to 94g•h^{-1} or 69% of daily CHO intake. Thus, for events such as marathons, ultramarathons, and multi-day cycling races, proper fueling with CHO during competition is extremely important to ensure success.

CHO Supplementation During Long Distance Endurance Exercise

Levine, et al. (1924) and Gordon et al. (1925) performed early research on CHO supplementation during exercise in a series of studies looking at the effects of long distance running on a number of physiological parameters. They found a close relationship between blood sugar levels and the athlete's physical condition following the race. Those with low blood sugar showed signs of shock, paleness, weakness, twitching, and even unconsciousness while those with normal blood glucose showed none of these adverse signs (Levine et al., 1924). They theorized that the hypoglycemic state could be attenuated by the ingestion of sugar during the race. The following year, before the 1925 Boston Marathon, several of the runners who had presented with post-race hypoglycemia in 1924 developed hypoglycemia during mile 14-18 of a training run. Because the authors had seen a correlation between low blood sugar and poor physical state (Levine et al., 1924), they had runners consume a high CHO diet prior to the race and candies containing 3g of glucose throughout the race (Gordon et al., 1925). All study participants had normal blood sugar levels following the race and showed none of the adverse signs seen the year before (Gordon et al., 1925). CHO supplementation during the race maintained blood glucose levels and prevented hypoglycemia, which allowed runners to finish in better physical condition.

The classic 1986 study by Coyle et al. (1986) demonstrated the importance of CHO ingestion during endurance exercise. Cyclists worked at 71 ± 1% $\dot{V}O_{2\,max}$ until exhaustion while consuming either a flavored placebo or glucose drink. Consuming CHO resulted in a 33% improvement in time to fatigue from 3.02 ± 0.19h to 4.02 ± 0.33h (Coyle et al., 1986). Muscle glycogen was utilized at similar rates for the first 3 hours in

both trials and no difference was found at the end of 3 hours. However, blood glucose levels were maintained in the CHO trial (4.2-5.2mM) and were significantly different from the placebo trial, which declined from 3.4 to 2.5mM from 80 minutes until the end of the trial (Coyle et al., 1986). From 80 minutes into the trial until the end, plasma free fatty acids were higher in the placebo group (Coyle et al., 1986). The results combine to show that CHO supplementation maintains blood glucose and reduces the use of fatty acids during prolonged exercise. The ability of participants in the CHO trial to maintain power output was accomplished through an elevated rate of CHO oxidation during the last 1.5h of the trial even though both trials showed equal rates of glycogen oxidation (Coyle et al., 1986). This study demonstrates that the ingestion of CHO improves endurance exercise performance through the maintenance of blood glucose levels and allowing for a continued high rate of CHO oxidation.

To determine how a sports drink effects exogenous, total CHO oxidation, and exercise performance, Roberts et al. (2014) compared the effects of a maltodextrin containing beverage to a maltodextrin and fructose drink. Participants worked at 50% of maximum power for 2.5h followed immediately by a 60km time trial (TT). Participants drank 270ml every 15min of either an artificially sweetened placebo, a drink that delivered 1.7g•min^{-1} maltodextrin (MD), or a drink delivering 1.1g•min^{-1} maltodextrin + 0.6g•min^{-1} fructose (MD + F). Just as Coyle et al. (1986) found, blood glucose levels were significantly higher in both CHO trials compared to the placebo (Roberts et al., 2014). During the placebo trial, 8 of the 14 participants were not able to even complete the 60km TT. Time to completion with MD + F compared to MD was reduced 7.2% and 6.5% compared to the placebo (Roberts et al., 2014). With regards to CHO utilization,

total CHO oxidation was greater in both CHO trials compared with the placebo, and during the last 30min of the 2.5h trial, MD + F showed significantly higher rates of total CHO oxidation than MD alone (Roberts et al., 2014). Exogenous CHO oxidation peaked in the final 30 minutes providing 1.45 ± 0.09g•min^{-1} and 1.07 ± 0.03g•min^{-1} of the total 2.81 ± 0.06g•min^{-1} and 2.42g•min^{-1} for MD + F and MD respectively (Roberts et al., 2014). It should be noted that the total CHO oxidation for the placebo trial during this time was only 2.00g•min^{-1} (Roberts et al., 2014). This study demonstrates that a CHO beverage with multiple types of CHO increases total CHO oxidation and spares endogenous CHO stores, while increasing power output compared to a CHO beverage with one type of CHO.

Undeniably, CHO supplementation during endurance activities lasting several hours is beneficial to the athlete through the maintenance of blood glucose levels and the possible sparing of muscle glycogen (Burke et al., 2011; Coggan & Coyle 1988; & Coyle et al., 1986). The American College of Sports Medicine Position Stand (Sawka et al., 2007) and Baker and Jeukendrup (2014) recommend consuming a fluid replacement beverage of 6-8% CHO such that 30-60g (Sawka et al., 2007) and up to 90g (Baker & Jeukendrup, 2014) are ingested per hour of exercise. They also recommend that several types of CHO be consumed to maximize total CHO absorption (Baker & Jeukendrup, 2014; Sawka et al., 2007). It is important for the athlete to train using CHO drinks if they plan to compete with them because at high intensities, sports drinks are associated with impaired gastric emptying and an increased chance of gastric distress (Baker & Jeukendrup, 2014; van Nieuwenhoven et al., 2004). Even with this side effect as a possibility, CHO are still recommended during prolonged activity.

The idea that the depletion of endogenous CHO stores is a limiting factor of exercise performance was termed the "energy supply/energy depletion model" of exercise fatigue by Dr. Timothy Noakes (2000). According to Noakes, hypoglycemia following endurance activity of sufficient length will limit performance because the liver's glycogen stores can no longer maintain blood glucose levels. The mechanism for fatigue in this case is not only that the muscle is incapable of producing sufficient ATP for contraction, but rather he suggests that central neural control could also limit the activity (Noakes, 2000). The brain is fueled by blood glucose. When hypoglycemia occurs, the brain loses its primary fuel source. But as Coyle et al. (1986) demonstrated, CHO supplementation maintained blood glucose levels during prolonged exercise while glycogen depletion leveled off. Noakes cites this as evidence that some other mechanism is at work that causes fatigue during prolonged exercise (Noakes, 2000). There is a clear relationship between low levels of endogenous CHO and fatigue which suggests that supplemental carbohydrate's ergogenic benefits during endurance performance is due to an increased rate of oxidation by the muscle itself. But, the idea that CHO work to preserve neural function during exercise cannot be overlooked.

CHO Supplementation During Exercise of 1h or Less

The fact that CHO may work through mechanisms other than preservation of blood glucose and muscle glycogen is seen in a number of studies that demonstrated a performance benefit when CHO were consumed during short duration, high-intensity activities. These studies showed that CHO ingested immediately prior to and/or during 1h of high-intensity cycling increased power output and total work completed (Jeukendrup et al., 1997; Anantaraman et al., 1995) and improved sprint performance (Ball et al.,

1995). Anantaraman et al. (1995) found that power output (W) was higher with CHO supplementation, but was only statistically significant during the last 20min of the 1h trial (168 ± 31W vs. 143 ± 29W) (Anantaraman et al., 1995). In Ball's investigation, maximum and mean power increased and fatigue rate decreased during a Wingate Anaerobic Power Test following 50min of cycling at 80% $\dot{V}O_{2\,max}$ (Ball et al., 1995). Jeukendrup et al. (1997) found that workload increased from 291.0 ± 10.3W to 297.5 ± 10.3W and time to completion of the trial improved from 60.15 ± 0.65min to 58.74 ± 0.52min with CHO consumption (Jeukendrup et al., 1997). Because they did not measure blood parameters, Jeukendrup et al. (1997) concluded "the explanation for this increased performance remains to be established." (p. 128)

CHO Mouth Rinses

Based on the conclusions by Below et al. (1995) who stated that CHO feeding did not exert its benefits through raising blood glucose or increasing CHO oxidation, Jeukendrup et al. (1997) suggested that CHO benefit the athlete through pathways other than energy production. These studies suggest that the ingestion of CHO provide an ergogenic benefit during high-intensity, short duration, exercise through different mechanisms than they might during long duration exercise. Previous research by Jeukendrup et al. (1997) and Carter et al. (2004b) found that direct infusion of CHO does not improve 1-hour exercise performance. Because of this, Carter et al. (2004a) suggested that during high-intensity exercise, CHO ingestion improves performance by increasing motor output through stimulation of oral receptors. Subjects in the study followed a similar protocol to that of Jeukendrup et al. (1997) but instead of consuming the CHO drink, the participants swished 25mL of a 6.4% non-sweet maltodextrin solution

every 12.5% of a set workload time trial following a 4h fast. The CHO beverage was rinsed for 5 seconds and expectorated. Compared to a water rinse, the CHO rinse trial decreased time to completion (61.37 ± 1.56min to 59.57 ± 1.50min) and increased power output (252 ± 16W to 259 ± 16W), while HR and rating of perceived exertion (RPE) increased throughout the trials but were not different (Carter et al., 2004a). During the first three quarters of the exercise trials, mean power output was higher in the CHO trial. Four of the nine subjects were able to distinguish the maltodextrin drink from the water control but their improvement was not different from the five who could not distinguish between the drinks (3.0 ± 3.8% vs. 2.9 ± 1.0%) (Carter et al., 2004a). These results suggest a "nonmetabolic" mechanism of performance enhancement that may work through oral receptors of the mouth. Since maltodextrin is not a sweet substance, the receptors responsible for stimulating this response are not the oral sweet receptors.

In a study of similar design to Carter et al. (2004), time to completion of a 1,000kJ time trial following a 4h fast was not statistically different when participants rinsed with CHO compared to no-rinse (65.7 ± 11.07min vs. 67.6 ± 12.68min) (Gam et al., 2013) but both trials were faster than a water rinse trial (69.4 ± 13.81 min) (Gam et al., 2013). Participants rinsed 25 ml of a 6.4% maltodextrin solution or 25ml of water every 12.5% of the trial. The authors hypothesized that the rinsing protocols interfered with the breathing pattern of the subjects and that more familiarization may lessen the interference. (Gam et al., 2013).

Sinclair et al. (2014) compared 5- and 10-sec rinses, hypothesizing that a longer rinse would activate more oral receptors and enhance performance to a greater degree than a 5-second rinse. During a 30min time trial, following a 4h fast, participants rinsed a

6.4% maltodextrin solution for 5 or 10sec every 6-min and found no significant difference in the cycling speed between the 5 (37.95 ± 3.95km•h^{-1}) and 10sec (38.66 ± 4.13km•h^{-1}) rinses. However, 8 of the 11 participants cycled further during the 10-second rinse trial (Sinclair et al., 2014). Even though the results suggest a 10sec rinse may be more beneficial, the authors suggest that during competitions or events where breathing rate is very high, a longer rinse may interfere with the athlete's breathing pattern (Sinclair et al., 2014). This becomes especially important during shorter, higher intensity events where breathing rates are increased. This study demonstrated the benefits of mouth rinsing as participants cycled farther with both CHO rinses compared to a water rinse (36.06 ± 4.4km•h^{-1}) (Sinclair et al., 2014). But the lack of a true placebo (i.e. an artificially sweetened rinse) may have influenced the study outcomes.

Time trials are an important discipline within cycling road racing, but success in races such as criteriums depends not only on a high power output throughout the race but also in a maximum effort sprint. To this end, Phillips et al. (2014) showed that following a series of 5-second CHO mouth rinses, peak power output increased 2.3% from 13.20 ± 2.14W•kg^{-1} to 13.51 ± 2.19W•kg^{-1}. Beaven et al. (2013) found similar improvements in sprint performance following just one, 5sec CHO mouth rinse. Peak power increased 22.1 ± 19.5W compared with the sprint following a placebo rinse. This study is limited, in that participants were recreationally trained, thus generalizing to highly trained athletes is restricted. In the Phillips et al. (2014) study, mouth rinses were administered during rest following a 2h fast and not during a period of exercise as would be experienced during a typical cycling race. But track cycling events may last only 30sec, in which case, this study demonstrated that a mouth rinse prior to those events may be beneficial. While

2.3% is a very small improvement, when considering that in professional cycling events winners are often determined by centimeters and milliseconds, a 2.3% difference could be the difference between first and second place. Both of these studies demonstrate improved sprint performance following a CHO mouth rinse.

A recent study by Jeffers et al. (2015) demonstrated an attenuation of neuromuscular fatigue but no performance improvement with a mouth rinse during a 1h cycling test composed of a 45min ride at 70% maximum power followed by a 15min TT. Average power was 248 ± 23W and 248 ± 39W for the CHO mouth rinse and placebo, respectively (Jeffers et al., 2015). Post-exercise knee extensor maximal voluntary contraction (MVC) was significantly reduced compared to baseline in both treatments with the placebo having a greater loss of MVC (20% ± 10%) (Jeffers et al., 2015) compared to the CHO mouth rinse (12% ± 8%) (Jeffers et al., 2015). The authors acknowledged that their findings are not in accord with the majority of the literature and suggested that the CHO mouth rinse time, exercise protocol, and nutritional state of the subjects in their own and the research noted above, may influence the outcomes of these studies (Jeffers et al., 2015).

CHO Mouth Rinses While Running

The first study to look at the effects of CHO mouth rinse on running performance found no difference in distance covered during a 45min running time trial when rinsing with a CHO solution (9333 ± 988m) compared to a 3% lemon juice placebo solution (9309 ± 993m) (Whitham & McKinney, 2007). Following a 4h fast, participants rinsed either a placebo or a 6% maltodextrin solution, both mixed with 3% unsweetened lemon juice every 6min during a 15min preload and the subsequent 45min time trial.

Participants were unaware of any performance data during the trials and were allowed to adjust the treadmill speed as they so choose throughout the trials. The authors suggested that the use of lemon juice to blind the participants to the solution contents was better than previously done (Whitham & McKinney, 2007).

The results of Whitman and McKinney (2007) are in contrast to a series of studies conducted by Rollo et al. (2008), Rollo et al. (2010), and Rollo et al. (2011). One of the challenges to studying running performance is that treadmills require manual changes to alter speed whereas cyclists can shift gears or increase cadence easily. Therefore, they made a treadmill that adjusted speed based on the runner's position on the treadmill thus eliminating the problem of manual input by the runner. Rollo et al. (2008) suggested that this limitation might explain why Whitham and McKinney (2007) found no differences in running performance when subjects were given a CHO mouth rinse. Rollo et al. (2008) investigated performance during a 30min run. HR, RPE, and gastrointestinal discomfort were not different between CHO rinse and placebo trials (Rollo et al., 2008). Following an overnight fast, participants rinsed with a 6% CHO beverage or a flavor matched placebo for 5-seconds immediately before and 3, 6 and 9.5min into a 10min warm up and every 5min during the 30min trial. Within the first 5 minutes of the trial, participants selected a faster pace when rinsing with CHO and maintained a higher speed throughout the trial, which resulted in running 115m farther, or a 1.7% improvement ($p<0.05$) (Rollo et al., 2008).

As a follow-up study, Rollo et al. (2010) investigated the effects of mouth rinsing on 1h run performance. Using the same treadmill as previously used (Rollo et al., 2008), participants were able to self-select their running speed based on their position on the

treadmill belt. Following a 13h fast, participants ran for an hour and rinsed with 25mL of a 6.4% CHO beverage or flavor matched placebo immediately before and every 15min during the trial. Participants ran 211m (1.5%) ($p<0.05$) farther while rinsing with the CHO beverage with no change in blood glucose or insulin levels (Rollo et al., 2010). Rollo et al. (2011) compared CHO mouth rinsing to CHO beverage ingestion during a 1h run following an overnight fast. During the ingestion trial, participants consumed approximately 60g of CHO before exercise, 25mL of the beverage immediately before the trial, and $2mL \cdot kg^{-1}$ of the beverage every 15min during the trial. This protocol was also completed with an artificially sweetened placebo beverage. Participants were also instructed to swish the final mouthful of beverage before swallowing. During the CHO mouth rinse trial, 25mL of the beverage was rinsed for 5sec, 30min before, immediately before, and every 15min during the trial. Participants ran farther using both the rinse and ingestion, but participants ran 230m ($p<0.05$) farther when ingesting the beverage compared to rinsing (Rollo et al., 2011). Thus the greatest benefit was seen with rinsing and ingestion of a CHO solution (Rollo et al., 2011).

Mechanism of Action

To investigate the hypothesis presented in these studies that CHO mouth rinses exert their ergogenic benefit through stimulation of oral receptors and subsequent activation of certain neural pathways, Chambers et al. (2009) used functional magnetic resonance imaging (fMRI) to examine brain regions activated with CHO mouth rinsing. Following an overnight or 6h fast, rinsing 25mL of a 6.4% glucose drink every 12.5% of a set amount of work time trial lowered time to completion (61.6 ± 3.8min to 60.4 ± 3.7min, respectively) with no difference in RPE (Chambers et al., 2009). A time trial

using maltodextrin yielded similar results and participants showed similar pacing strategies. The fMRI showed only small differences in brain activation when given glucose compared to maltodextrin, with similarity in activation of the primary and secondary taste cortex, prefrontal cortex, the right caudate, and the ventral striatum among others which mediate behavioral and emotional responses to taste and control motor behavior, (Chambers et al., 2009). It was thought that a combination of sweet and somatosensory receptors are responsible for the response due to the maltodextrin's low concentration of mono- and disaccharides (~1.6%). The ventral striatium, part of the dopaminergic system, is associated with motor control and motivation. As Noakes' (2004) "Central Governor Model" would suggest, fatigue is (at least in part) due to the brain limiting motor output. By rinsing the mouth with CHO, the inhibition of the dopaminergic pathways is minimized and, thus, the feelings of fatigue are reduced. The authors suggest "that this is the mode of action of oral CHO," (Chambers et al., 2009, p. 1792) which is similar to that seen with the administration of drugs like caffeine and methamphetamines.

CHO sensed in the mouth provide a very rapid ergogenic benefit (Gant et al., 2010). Immediately after ingesting a CHO drink, maximum voluntary contraction (MVC) is increased compared to a placebo and motor evoked potential (MEP) amplitude from the right first dorsal interosseous is increased (Gant et al., 2010). Oral receptors transmit signals through cranial nerves VII, IX, and X to the medulla and pons, which can modulate motor output (Gant et al., 2010). Because both the placebo and CHO drink had equal amounts of artificial sweetener, the authors suggested that unidentified receptors sense the nutrient density of the maltodextrin and facilitate the increase in MEP (Gant et

al., 2010). Areas of the brain associated with movement, specifically the contralateral sensorimotor cortex and ipsilateral cerebellum, experienced greater activation during a pinch-grip test when CHO were present in the mouth (Turner et al., 2014). As well, CHO facilitated activation of areas of the brain responsible for information processing in the visual centers and the anterior cingulate gyrus, which is associated with emotional responses to food (Turner et al., 2014). During times of rest, motor activity was not different, suggesting that the effects of mouth rinsing may not be seen when energy expenditure is low (Turner et al., 2014). These studies together demonstrate that there are neural pathways that sense CHO in the mouth that stimulate reward and motor areas of the brain which can attenuate the drop in motor output during exercise.

The idea that receptors in the mouth stimulate motor output through sensation of CHO nutrient density suggest that time trial performance will be higher with a higher concentration of CHO in the mouth rinse. If CHO nutrient density is not a key factor contributing to motor output, and simply the sensation of any CHO in the mouth produces a motor response, no improvement would be expected when using different mouth rinse concentrations.

CHO Mouth Rinse Dose Response

Only three studies have investigated the effects of CHO mouth rinse concentration on exercise performance. Wright and Davison (2013) showed an increase in running distance during a 90-minute treadmill run following a 6h fast with both a 6% (14.6 ± 1.7km) and 12% (14.9 ± 1.6km) CHO mouth rinse, compared with an artificially sweetened placebo (13.9 ± 1.7km) (Wright & Davison, 2013). In all three trials, participants rinsed for 5sec immediately before and every 15min during the trial until

minute 45. While both CHO mouth rinses improved running performance compared to the placebo there was no difference between the 6% and 12% CHO mouth rinses ($p = 0.196$) (Wright & Davison, 2013).

Similarly, an abstract by Bottoms et al. (2014) found no difference between a, 0%, 3%, 6%, and 12% CHO mouth rinses during a 30-minute cycling time trial but they do not state the frequency of mouth rinse delivery. Distance covered was 16.6 ± 3.0km, 16.5 ± 2.9km, 16.6 ± 3.2km, and 16.5 ± 2.7km, respectively (Bottoms et al. 2014). While performance was not different, cadence, speed, and power differed between trials suggesting that the rinse concentration affected participant pacing strategy but the abstract does not describe how (Bottoms et al. 2014).

Ispoglou et al. (2015) found that following a 3h fast, 1h cycling time trial performance was not improved compared to a placebo (251 ± 28W) with a 4% (248 ± 28W), 6% (246 ± 31W), or 8% (247 ± 33W) CHO mouth rinse (Ispoglou et al., 2015). Blood glucose, blood lactate, HR, RER, CHO oxidation, or RPE were also not different between trials (Ispoglou et al., 2015). During each trial, participants rinsed 25ml of the experimental solution for 5sec every 12.5% of the trial. The authors suggested that the increased perceived thirst during CHO trials and the lack of fluid intake may have influenced the results even though no relation between thirst and power was found (Ispoglou et al., 2015). Because of the protocol differences of rinse duration, concentration and frequency and small sample size it is difficult to draw conclusions regarding the effects of CHO mouth rinse concentration on exercise performance.

Prandial State

It appears from the research that the prandial state of the athlete may determine the effectiveness of a CHO mouth rinse. When fed a meal two hours before the start of a time trial, mouth rinsing a 6.4% maltodextrin solution for 5sec every 12.5% of the trial had no effect on time trial performance (Beelen et al., 2009). Average power during CHO and placebo trial was 265 ± 5W and 266 ± 5W and time to complete a set amount of work was 68.14 ± 1.14min and 67.52 ± 1.0min, respectively (Beelen et al., 2009). Both HR and RPE were not different between trials (Beelen et al., 2009). Similar results were not seen in a study using nonathletic participants (Fares & Kayser, 2011). Time to exhaustion in nonathletic subjects was increased with a 5-10sec 6.4% maltodextrin mouth rinse every 5min (56.6 ± 12.2min) compared to a placebo (54.7 ± 11.3min) in both a fed state and rinsing a CHO solution (53.9 ± 12.8min) compared to a placebo (48.3 ± 15.3min) following an overnight fast (Fares & Kayser, 2011). In a fed state, mean RPE was reduced from 5.5 ± 0.7 to 5.0 ± 0.7 as well as maximum RPE from 8.9 ± 0.4 to 8.6 ± 0.5 (Fares & Kayser, 2011). Neither mouth rinse or prandial state had an effect on average HR but there was a significant interaction. The authors noted that this might be due to chance because of the variability seen (Fares & Kayser, 2011).

In a hot and humid environment during Ramadan fasting, Muhamed et al. (2014) showed a CHO mouth rinse improved 10km cycling time trial performance following 30 minutes of preload exercise from 16.8 ± 1.6min to 12.9 ± 1.7min compared to not rinsing at all with a reduction in final RPE from 8 ± 1 to 6 ± 2. However, there were no differences between time trial performance and RPE when rinsing with CHO compared

to the placebo (12.9 ± 1.7min vs. 12.6 ±1.7min; RPE 6 ± 2 vs. 7 ±2) (Muhamed et al. 2014).

Ataide-Silva et al. (2016) showed a performance improvement with a CHO mouth rinse when participants were in a CHO depleted state following exhaustive exercise and an overnight fast. Time to complete a 20km cycling time trial was reduced with CHO mouth rinse while RPE did not change (Ataide-Silva et al., 2016). Similarly, Lane et al. (2013) showed that after an overnight fast, a 10% CHO mouth rinse solution immediately before and 12.5% of a 1h cycling time trial improves cycling time trial performance by 3.4% (273 ± 6W vs. 282 ± 6W) whereas performance during a time trial 2h after a meal was improved only 1.8% (281 ± 5W vs. 286 ± 6W) with a mouth rinse. Lane et al. (2013) concluded that in a fasted state, CHO mouth rinsing improves performance to a similar degree as consuming a CHO rich meal prior to exercise. For athletes who suffer GI distress due to meal consumption prior to a competition, CHO mouth rinse offers an alternative strategy to maximize performance.

Summary

Performance in endurance sports is affected by a number of variables but the ability of the athlete to maintain a high percentage of their $\dot{V}O_{2\,max}$ is critical for success. During events that exceed 90min, CHO supplementation becomes necessary to slow the decay in power. CHO supplementation maintains blood glucose levels and possibly spares muscle glycogen. During hour-long events, CHO supplementation similarly improves performance but without any changes in blood glucose levels or exogenous CHO oxidation rates. Carter et al. (2004a) was the first to propose that during these events, CHO are sensed in the mouth and exert their ergogenic effect through the central

nervous system. Indeed, mouth rinsing activates centers of brain associated with reward and motor output. Although the exact mechanism has yet to be identified, a majority of studies show that using a CHO mouth rinse is beneficial during endurance exercise lasting approximately 1h. Using a mouth rinse during high intensity endurance exercise may also eliminate the gastrointestinal distress many athletes suffer from during these events.

CHAPTER THREE: METHODS

Participants

Participants were recruited from the Boise State University campus and local community for this study. Eligibility criteria included: male, 18-45 years old, average of 30 miles of cycling per week during the previous 6 months, and free of known cardiovascular or metabolic diseases. Only men were included due to the CHO metabolic changes that occur during different phases of the menstrual cycle (Webb, 1986; Solomon et al., 1982). This study was approved by the university's Institutional Review Board. Prior to enrollment, participants completed a health and exercise history questionnaire (Appendix A) and sign an informed consent form (Appendix B).

Procedures

Overview

Participants completed a familiarization trial and four experimental trials: rinsing with a 0%, 3%, 6%, or 12% CHO solution. Each visit occurred at approximately the same time of day and was separated by a minimum of five days to ensure adequate recovery time between trials. The familiarization trial was identical to the experimental trials with the exception of a water rinse instead of a CHO rinse. After completing the familiarization trial, participants were given a diet and exercise log and asked to complete it for 3 days prior to the first experimental trial. Participants were asked to maintain that same diet and training schedules prior to all experimental trials to minimize differences in muscle glycogen content and energy availability. Participants were also instructed to

refrain from vigorous exercise and alcohol for 24h prior to each visit, and caffeine for 6h prior to each visit.

Experimental Procedures

Baseline Measurements

Body composition was assessed during the first visit using a BOD POD (Life Measurement Inc., Concord, CA). Participants were instructed to wear lycra or spandex clothing for measurement of body mass and body composition. Two to three body volume assessments were completed and the average was used to calculate body density and subsequent calculation of body composition. Upon completion, the participant changed into comfortable cycling apparel and shoes.

Cycle Ergometer Time-Trials

Each participant completed four 1h cycling time trials during which they were asked to complete as much work as possible. All trials were conducted on a Velotron Pro (Racermate Inc., Seattle, WA) cycle ergometer that was adjusted such that saddle height, saddle fore-aft position, handlebar height, and handlebar fore-aft position were comfortable for the participant. Ergometer setup was recorded during the familiarization trial and used during each subsequent visit. Room temperature and atmospheric pressure were recorded at the start of each trial. Urine specific gravity was determined using a refractometer (ATAGO U.S.A., Inc., Bellevue, WA) before each trial to ensure adequate baseline hydration. Participant weight was recorded before and after each trial to assess total fluid loss.

For all trials, participants performed a 10min warm up at a self-selected work rate and cadence followed immediately by a one-hour time trial at a self-selected work rate

and cadence. The time trial began at 50W at a self-selected cadence and participants were instructed to adjust the resistance using a control box mounted to the cycle ergometer handlebar. Heart rate (HR) was continuously monitored using a Polar Electro Oy (Kempele, Finland) heart rate monitor (Goodie et al., 2000).

Performance was measured at each 15min time point as work performed (kCal) and average power (W) for the trial. Participants were unaware of the time until minute 55 at which time they were made aware that 5min remained in the trial. No other verbal encouragement or feedback was given to the participant during the trial. Study related variables were not revealed to the participant for any trial until after the completion of the final experimental trial. A fan was placed on a table 1.5m in front of the participant to provide cooling. Rating of perceived exertion (RPE) and HR were also recorded every 15min during each time trial.

CHO Mouth Rinse

A third party who was not involved in the data collection prepared all mouth rinses prior to the arrival of the participant. Rinses were prepared using a commercially available CHO-electrolyte (CE) drink powder made with sucrose or a CHO-free, flavor matched sucralose-sweetened liquid electrolyte concentrate as the placebo (The Coca-Cola Co, Atlanta, GA). The placebo concentrate was mixed with 8 fluid ounces of water to yield a CHO-free solution. The 3, 6 or 12% CHO solution was made similarly with 8 fluid ounces of water and the CE powder. A 25mL sample of the solution was measured and transferred into 4 fluid ounce flasks and refrigerated prior to each trial. Nutritional information for each rinse are presented in Table 3.1.

Table 3.1. Nutritional Content of Each 25ml Rinse

	Placebo	3%	6%	12%
Kcal	0	2.8	5.6	11.2
CHO (g)	0	0.7	1.4	2.8
Sodium (mg)	10.6	5.3	10.6	21.2
Potassium (mg)	2.6	1.2	2.5	4.9

Trial order was randomly assigned for each participant using a balanced randomization scheme. Mouth rinses were given in a small opaque bottle to swish in the participant's mouth for 5sec before expectorating the rinse into a measuring cup at time 0, 15, 30, and 45min of the time-trial. After each rinse, any difference in mouth rinse volume was measured to determine if any of the rinse had been swallowed. Rinse conditions were administered in a double blind fashion during each trial. Following the final experimental trial, participants were asked to recall the order in which they thought they received the rinses.

$\dot{V}O_{2\,max}$ Test

Maximal oxygen consumption ($\dot{V}O_{2\,max}$) was assessed during a continuous, incremental exercise test on a cycle ergometer using the Parvo Medics TrueOne® 2400 pneumotach metabolic cart (Sandy, UT) to determine the cardiorespiratory fitness level of participants. Participants were given a 10min warm-up on the cycle ergometer at a self-selected pace. Participants began the test pedaling at 50W for 2min and increased 50W every two minute until 200W was reached after which resistance was increased by 25W every minute until the participant reached volitional exhaustion (Bassett & Howley,

2000). This protocol was modified for participants such that they would finish in less than 15min. Expired air was collected through a Hans Rudolph one-way valve and mouthpiece connected to the metabolic cart for gas analysis. HR was continuously monitored. Participants were verbally encouraged throughout the test. Criterion for a maximal effort included at least one of the following: Respiratory Exchange Ratio greater than or equal to 1.10, oxygen consumption plateau, or a heart rate within 10-12 beats of age predicted maximum (Howley, Bassett, & Welch, 1995; Beam & Adams, 2014). Participants were instructed to remain seated throughout the test and maintain a self-selected cadence greater than 60RPM until volitional fatigue.

Data Analysis

Data were reported as means and standard deviations (mean ± SD). The dependent variables (work completed (kCal), average power (W), average heart rate, average cadence, and RPE) at each time point for each condition were compared using repeated-measures analysis of variance (ANOVA). When the assumption of sphericity was violated, Greenhouse Geiser adjustment was applied to the ANOVA p-value indicated in the text with the subscript $_{GH}$ following the p-value. If necessary, post-hoc pairwise comparisons with Bonferroni adjustments were performed to detect variable differences between trials. Statistical significance was set at alpha of 0.05. All data was analyzed using SPSS (version 22.0, Chicago, IL).

CHAPTER FOUR: RESULTS

A total of fifteen participants enrolled in this study. One participant withdrew for personal reasons after completing one of the four experimental trials. Thus, analysis was completed for the fourteen participants that completed all phases of the project. Participant demographics are presented in Table 4.1. All but one participant had previous racing experience. Six participants reported that they participated in primarily road cycling, four in triathlons, three in both mountain and road, and one in mountain biking only. The cycling experience of the participants is presented in Table 4.2.

Table 4.1. Participant demographics, *n*=14 unless otherwise noted

Age (y)	30.2 ± 7.2
Height (m)	1.81 ± 0.07
Mass (kg)	75.7 ± 11.0
BMI ($kg \cdot m^{-2}$)	23.0 ± 2.4
Body Fat %	11.6 ± 4.5
Absolute $\dot{V}O_{2max}$ ($L \cdot min^{-1}$) (*n*= 9)	4.4 ± 0.89
Relative $\dot{V}O_{2max}$ ($ml \cdot min^{-1} \cdot kg^{-1}$) (*n*= 9)	59.5 ± 7.2
Max HR (*n*= 8)	181 ± 8.9

Table 4.2. Participant cycling experience

Years of Cycling	7.6 ± 5.9
Avg Rides Per Week	4.75 ±1.7
Avg Miles Per Ride	25.1 ±14.9
Avg Miles Per Week	110.5 ± 66.0

Room temperature and barometric pressure were not significantly different between trials (See Appendix C). Participant USG and percentage change in body weight were not significantly different between trials (See Appendix D). Percentage weight loss within trials was statistically different from 0 ($p < 0.01$).

Repeated measures ANOVA revealed no significant main effect of CHO mouth rinse concentration on total work completed, expressed as cumulative calories burned ($p = 0.405$) or mouth rinse concentration*time interaction ($p = 0.708$). Mean calories burned during each trial are presented in Figure 4.1. There was no significant main effect of mouth rinse concentration on calories burned per quarter (15min) of each trial ($p = 0.420$) or mouth rinse concentration*time interaction ($p = 0.465$). Calories burned per quarter of each trial are presented in Figure 4.2.

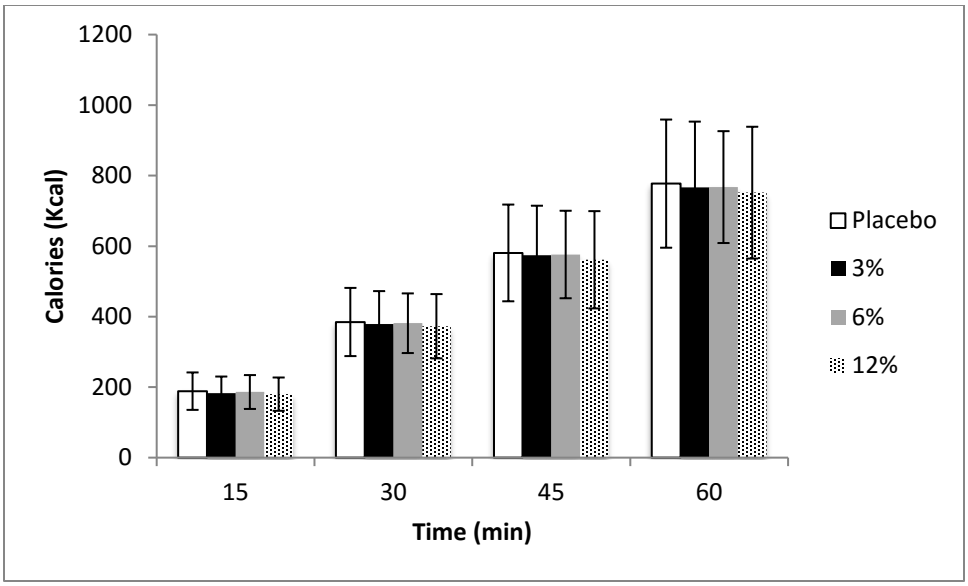

Figure 4.1. Average Values for Cumulative Calories Expended Per Trial

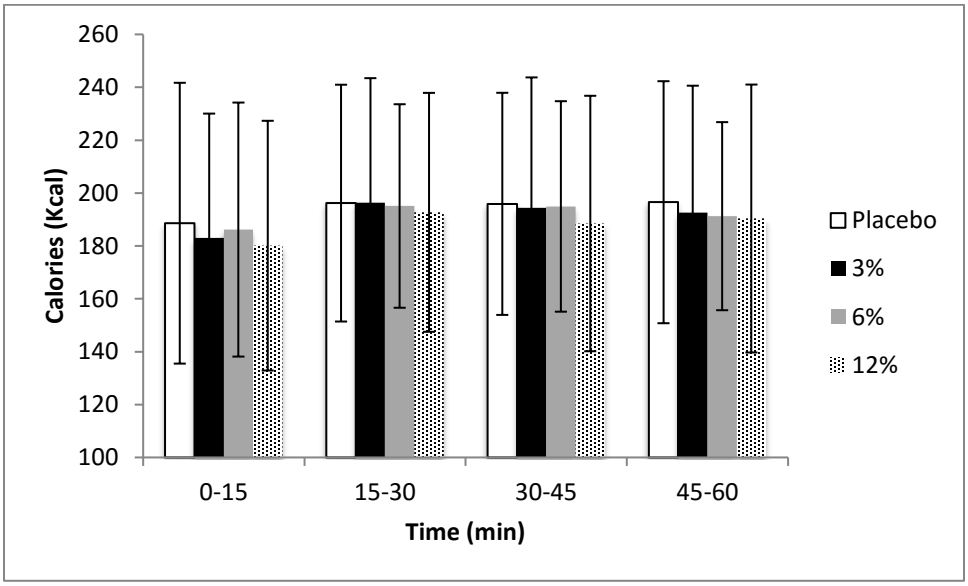

Figure 4.2. Average Values for Calories Expended per Quarter of Trial

There was no significant difference in average power between trials ($p = 0.082$) and no significant mouth rinse concentration*time interaction ($p = 0.167$). Average power data is presented in Figure 4.3.

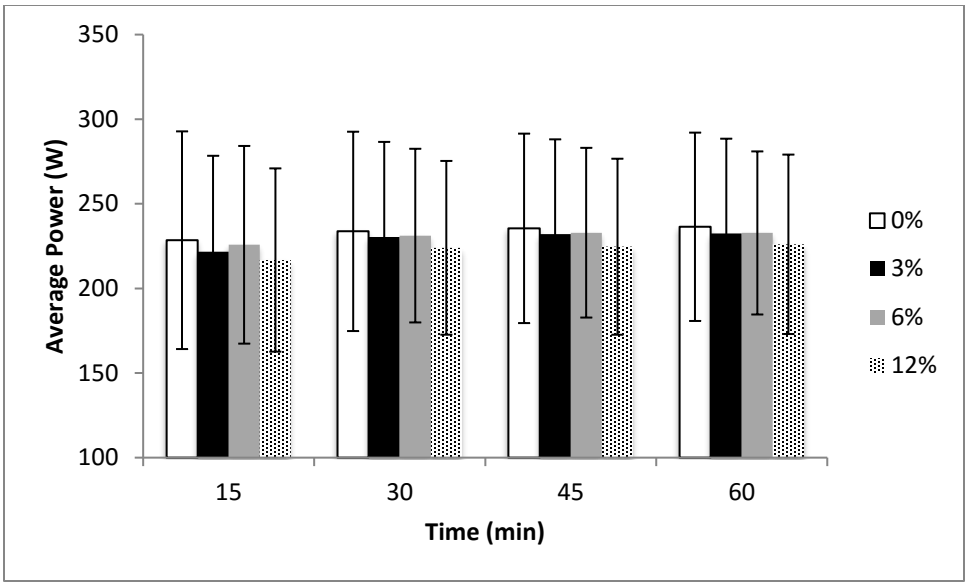

Figure 4.3. Average Power during Each Trial

There was a significant main effect of time on RPE ($p < 0.001$). RPE increased throughout each trial with RPE at each time point being higher than the previous (all $p < 0.01$). There was no significant main effect of mouth rinse concentration on RPE ($p = 0.704_{GH}$) or mouth rinse concentration*time interaction ($p = 0.670$). RPE data is presented in Figure 4.4.

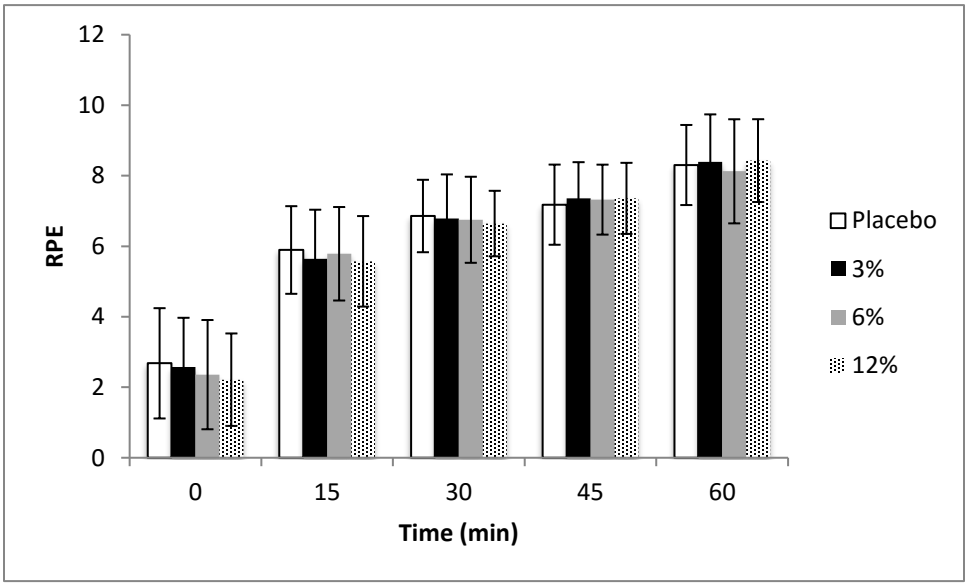

Figure 4.4. Rating of Perceived Exertion during Each Trial

There was a main effect of time on average HR (beats per minute) ($p < 0.001$). Average HR increased throughout each trial and each time point was significantly higher than the previous time point (all $p < 0.01$). There was no significant main effect of CHO mouth rinse concentration on HR ($p = 0.399$) or mouth rinse concentration*time interaction ($p = 0.818$). Percentage of maximum HR was calculated for those that performed a $\dot{V}O_{2max}$. There was no main effect of condition ($p = 0.518$). For the entire trial, participants HR averaged 87.7% of their maximum. Average HR data is presented in Figure 4.5.

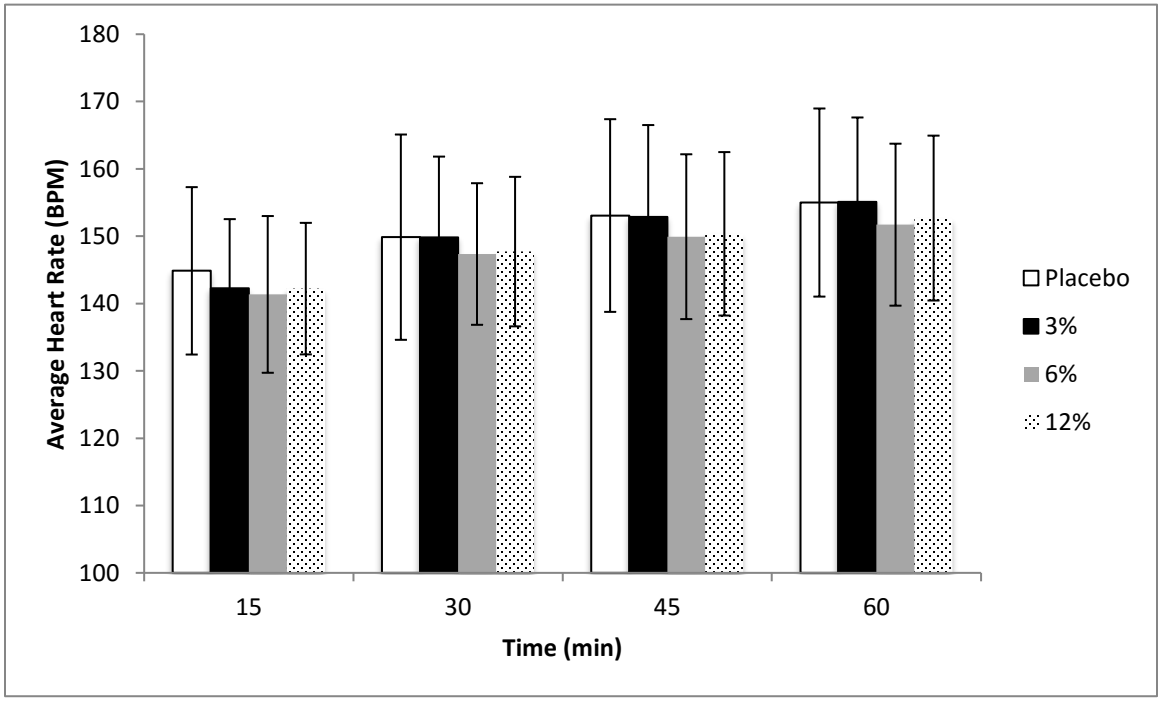

Figure 4.5. Average Heart Rate during Each Trial

There was no significant main effect of mouth rinse concentration (p = 0.640), time (p = 0.359), or mouth rinse concentration*time interaction (p = 0.131) for average cadence. Cadence data is presented in Figure 4.6.

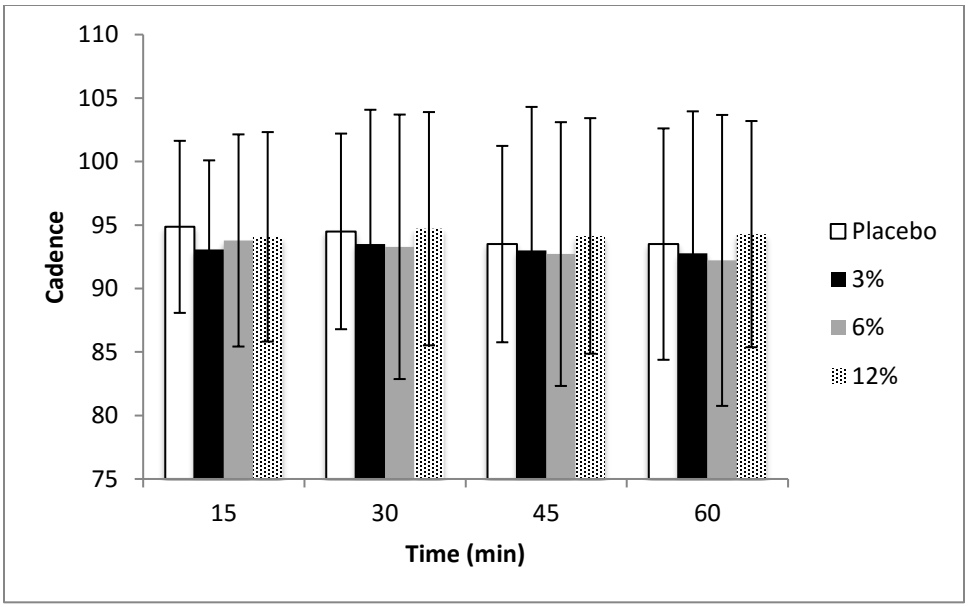

Figure 4.6. Average Cadence during Each Trial

CHAPTER FIVE: DISCUSSION

The purpose of this study was to determine if a dose-response relationship exists between CHO mouth rinse concentration and 1h cycling time trial performance. We hypothesized that there would be a dose-response effect to CHO mouth rinse concentration. The results of the present study found that 1h cycling time trial performance, measured as both trial average power and work completed was not different when rinsing with a 0%, 3%, 6%, or 12% CHO solution, which did not support our hypothesis. These results indicate that there is no ergogenic benefit of a CHO mouth rinse for endurance performance of 1h in duration.

This is the fourth study to investigate the effects of CHO mouth rinse concentration on exercise performance. Our results are in agreement with Bottoms et al. (2014), Ispoglou et al. (2015), and Wright and Davison (2013) that a dose response to CHO mouth rinse does not exist. Bottoms et al (2014) showed that distance covered during a 30min cycling time trial was not statistically different when participants rinsed with a 0%, 3%, 6%, and 12% CHO mouth rinse. Ispoglou et al. (2015) showed no difference in 1h cycling time trial performance with a 4%, 6%, or 8% CHO mouth rinse. In the present study, average power, work completed, RPE, and HR during a 1h time trial were not different when rinsing with different CHO mouth rinse concentrations.

While Wright and Davison (2013) did not demonstrate a dose-response to CHO mouth rinse concentration, they did show a performance improvement with a 6% and 12% CHO mouth rinse compared to a 0% CHO placebo rinse. However, the results of the present study are not supported by previous research that demonstrates the ergogenic

effects of using a CHO mouth rinse. It has been proposed that performance improvements when using a CHO mouth rinse during exercise are principally seen when the athlete is fasted (Ataide-Silva et al., 2016; Muhamed et al., 2014; Fares & Kayser, 2011; Ispoglou et al., 2015; Lane et al., 2013). Time to exhaustion increased by 7% following an overnight fast and by 3% three hours after a meal was consumed (Fares & Kayser, 2011). Lane et al. (2013) found a statistically significant 3.4% improvement in cycling time trial performance with a CHO mouth rinse in a fasted state compared to a statistically significant 1.8% improvement two hours after consuming a meal. However, Ispolgou et al. (2015) showed no statistical difference in performance during a 1h cycling time trial when rinsing with a 4%, 6%, or 8% CHO mouth rinse when participants had consumed a meal three hours prior to beginning the trial. Muhamed et al. (2014) found that time trial performance improved with both a placebo and CHO mouth rinse compared to not rinsing when participants were fasted during Ramadan. Lastly, Ataide-Silva et al. (2016) found that following a muscle glycogen depleting bout of exercise and subsequent overnight fast, CHO mouth rinse restored EMG activity of the vastus lateralis and improved cycling time trial performance compared to a placebo. These studies demonstrated that athletes in a fasted state may best utilize a CHO mouth rinse. The results of Ispoglou et al. (2015) that there are no CHO mouth rinse dose response are in agreement with the findings of the present study. However, in the present study, we did not control for the timing of the final, pre-time trial meal which may influence the effectiveness of a CHO mouth rinse as demonstrated in the above studies (Ataide-Silva et al., 2016; Fares & Kayser, 2011).

Examining studies demonstrating performance improvements with a CHO mouth rinse shows the importance of prandial state on the effectiveness of using a CHO mouth rinse. Following a 2h fast, repeated sprint performance was improved (Beaven et al., 2013) and peak power output increased (Philips et al., 2014). Following a 4h fast, time trial performance (Carter et al., 2004a) and distance cycled improved (Sinclair et al., 2014) Following a 10h fast, MEP increased 30% and MVC increased 2% (Gant et al., 2010). Following a 13-15h fast, participants ran 211m farther (Rollo et al., 2010). Following a 6h or overnight fast, participants cycled faster (Chambers et al., 2009). And lastly, following an overnight fast, torque attenuation was decreased (Jensen et al., 2015). Considering the studies cited above and those that directly examined CHO mouth rinse effects in pre- and postprandial states, it appears that CHO mouth rinsing is more beneficial in the postprandial state. However, participants exercised 3h after a meal (Ispoglou et al., 2015), 4h after a meal (Whitman & McKinney, 2007), and following an overnight fast (Watson et al., 2014) and no improvement was seen with a CHO mouth rinse. The discrepancy between Watson et al. (2014) and other research may be due to the heat stress during trials, which minimized the effectiveness of a CHO mouth rinse. In the present study, participants followed the same three-day diet prior to each time trial, but the timing of the last meal before each trial was not controlled for. Therefore, the lack of improvement in performance between the placebo and CHO rinse trials may have been due to the prandial state of the participant.

Neural response to tasting CHO has been shown to differ based on the level of satiety of the individual. Haase et al. (2009) showed significant difference in neural response in the insula, thalamus, hippocampus, amygdala, and anterior cingulate gyrus.

These regions are associated with metabolic processes and anticipation of rewarding stimuli (Haase et al., 2009). Oral sensation of sucrose produced significantly greater neural activation compared to caffeine, citric acid, guanosine 5'- monophosphate, saccharin, and sodium chloride – particularly in a hunger state (Haase et al., 2009). This demonstrates the change in response to CHO in the mouth during different states of hunger: in a hunger state, an individual may have a greater sensation of reward value from CHO in the mouth compared to a satiated state (Haase et al., 2008). The consumption of food prior to exercise may minimize the rewarding stimuli of CHO in the mouth and explain the lack of improvement seen in present study when CHO were rinsed in the mouth during exercise.

Several other factors may have influenced the outcome of the present study. Participants consistently complained of being thirsty during the trials. However, average percentage weight loss due to sweat during each trial was less than 2% and participants who lost greater than 2% body weight did so consistently across trials. Weight loss greater than 2% is when endurance exercise performance is compromised due to dehydration (Sawka et al., 2007). The type of time trial employed may also have affected participant motivation to complete each trial at a maximal effort. A time trial of a set amount of work may encourage participants to work harder knowing that the harder they work, the sooner they will be done whereas during a 1h time trial, the participant knows they have to work for an hour regardless. Even if the type of time trial did not directly influence participant motivation, it is difficult to assess participant motivation levels during the trials. With regards to the participants chosen to study, differences in the effectiveness of a CHO mouth rinse with regard to the training level of the athlete have

not been investigated. We chose to recruit participants with cycling experience so they would have an understanding of pacing strategy for a 1h effort. Previous studies have used untrained, recreationally trained, and endurance trained participants (Burke & Maughan, 2015). Lastly, the CHO in the CHO beverage used in the present study was sucrose. There is no difference in endurance performance when using sucrose, glucose, or maltodextrin (Massicotte et al., 1989; Murray et al., 1989) but because the oral receptor responsible for initiating the neural response to CHO in the mouth has yet to be determined, it may be that the type of CHO plays a role in the ergogenic effects of a CHO mouth rinse.

This study had several strengths. This study had a larger sample size (n=14) than other CHO mouth rinse dose-response studies. Of the studies cited investigating the effects of CHO mouth rinse concentration on exercise performance, Wright and Davison (2013) and Ispoglou et al. (2015) had seven participants each and Bottoms et al. (2014) had thirteen participants. Another strength was the double blind study design. Based on feedback from participants, the blinding scheme was effective. Only one participant correctly identified the placebo, five identified the 3% CHO rinse, three correctly identified the 6% rinse, and 5 correctly identified the 12% rinse. Only one participant correctly identified all four rinses. While a placebo effect may have affected some individuals, participants, in general, could not accurately distinguish the order that rinses were administered. Finally, participants were required to complete a full 1h familiarization trial allowing them to become accustomed to the environmental conditions as well as ergometer control and required effort. The familiarization trial

limits any learning effect that may have occurred across trials (Barfield et al., 2002; Ozkaya, 2013).

A possible limitation was not controlling for the timing of the pre-trial meal. Also, the control of the ergometer through manually adjustment of the resistance may inhibit the participants from making small, subconscious adjustments to their pace. This is different from cycling outdoors where participants are accustomed to adjusting resistance through shifting gears. However, participants were free to pedal at a self-selected cadence allowing for minor changes in pace that would occur normally during outdoor cycling. They were also free to sit or stand allowing for the simulation of outdoor cycling. The selection of an indoor cycling ergometer is justified in that it allowed for the control of environmental factors such as changes in surface gradient, temperature, wind, and barometric pressure.

Future Areas of Research

CHO mouth rinsing is generally accepted in the scientific literature as a beneficial practice during exercise of approximately 1h but the variety of protocols employed makes it difficult to know when to employ a CHO mouth rinse (De Ataide e Silva et al., 2014). Future research investigating the effects of CHO mouth rinse concentration on exercise performance should control for the pre-exercise diet such that participants complete each trial following a fast. If fasting is indeed a prerequisite to seeing performance improvements when using a CHO mouth rinse, then as Lane et al. (2013) suggests the length of fast prior to exercise that is required to see a benefit from CHO mouth rinsing should be determined. As well, future research should investigate the effect of CHO mouth rinse during exercise in trained vs. untrained athletes and women.

Conclusions

This study found no performance improvement when using a CHO mouth rinse. These results are in contrast to previously published literature that found a significant performance improvement when using a CHO mouth rinse which may be due to the prandial state of the athlete prior to exercise. The results also found no dose-response relationship between CHO mouth rinse concentration and 1h cycling time trial performance confirming results from previous research (Bottoms et al., 2014; Ispoglou et al., 2015; Wright & Davison, 2013). Further research is warranted to determine if a dose response to CHO mouth rinse exists during exercise in a fasted state.

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APPENDIX A

Institutional Review Board Approval Letter

BOISE STATE UNIVERSITY
RESEARCH AND ECONOMIC DEVELOPMENT

Date: August 13, 2015 **To:** Jonathan Youell cc: Scott A. Conger

From: Biomedical Institutional Review Board (MED-IRB) c/o Office of Research Compliance (ORC)

Subject: MED-IRB Notification of Approval - Original - 103-MED15-004 *Effects of Carbohydrate Mouth Rinse Concentration on Cycling Time Trial Performance*

The Boise State University IRB has approved your protocol submission. Your protocol is in compliance with this institution's Federal Wide Assurance (#0000097) and the DHHS Regulations for the Protection of Human Subjects (45 CFR 46).

Protocol Number: 103-MED15-004 Received: 7/6/2015 **Expires: 8/12/2016** Approved: 8/13/2015

Review: Expedited Category: 4, 7

Your approved protocol is effective until 8/12/2016. To remain open, your protocol must be renewed on an annual basis and cannot be renewed beyond 8/12/2018. For the activities to continue beyond 8/12/2018, a new protocol application must be submitted.

ORC will notify you of the protocol's upcoming expiration roughly 30 days prior to 8/12/2016. You, as the PI, have the primary responsibility to ensure any forms are submitted in a timely manner for the approved activities to continue. If the protocol is not renewed before 8/12/2016, the protocol will be closed. If you wish to continue the activities after the protocol is closed, you must submit a new protocol application for MED-IRB review and approval.

You must notify the MED-IRB of any additions or changes to your approved protocol using a Modification Form. The MED-IRB must review and approve the modifications before they can begin. When your activities are complete or discontinued, please submit a Final Report. An executive summary or other documents with the results of the research may be included.

All forms are available on the ORC website at http://goo.gl/D2FYTV Please direct any questions or concerns to ORC at 426-5401 or humansubjects@boisestate.edu. Thank you and good luck with your research.

Dr. Cheryl Jorcyk

Chair Boise State University Biomedical Institutional Review Board

1910 University Drive Boise, Idaho 83725-1139
Phone (208) 426-5401 orc@boisestate.edu

APPENDIX B

Health and Exercise History Questionnaire

Subject Number: _____ Test Date: _____

HEALTH AND EXERCISE HISTORY QUESTIONNAIRE

NAME: _____ _____ AGE: _____ DATE OF BIRTH:_____
First Last

ADDRESS: _____
Street City State Zip

TELEPHONE: _____ E-mail address: _____

Person to contact in case of an emergency: _____ Phone # _____
(relationship) _____

Has your physician ever told you that you have any of the following? (Yes or No)

YES	**NO**		**If yes, explain:**
_____	_____	Any heart trouble	_____
_____	_____	Diabetes	_____
_____	_____	Stroke	_____
_____	_____	Heart murmur	_____
_____	_____	High Blood Pressure	_____
_____	_____	Seizures	_____
_____	_____	Thyroid disorders	_____

In the past 30 days, have you had any of the following? (Yes or No)

YES	**NO**		**If yes, explain:**
_____	_____	Chest Pain	_____
_____	_____	Shortness of breath	_____

In the past 30 days, have you had any of the following? (Yes or No)

YES	**NO**		**If yes, explain:**
_____	_____	Feeling faint/dizzy	_____ ___
_____	_____	Heart palpitations	_____ __
_____	_____	Severe Headache	_____
_____	_____	Hospital admission	_____ _____

Are you taking any prescription or over-the counter medications? Yes ___ No ___

Name of medication Re ason for Taking F or How Long?

_____

_____

_____ _____

Do you currently engage in cycling on a regular basis? Yes ____ No ____

How many days per week? _____

How many miles do you ride per session? _____ per week?_____

How long have you been engaged in cycling training? _____

What is your main cycling discipline (Road, mtn, track, triathlon)? _____

Do you or have you participated in organized races? Yes ____ No ____

Do you ever have an uncomfortable shortness of breath during exercise? Yes ____ No ____

Do you ever have chest discomfort during exercise? Yes ___ No ___

FOR STAFF USE:

_____

_____ _____

_____

_____

APPENDIX C

Informed Consent Form

BOISE STATE UNIVERSITY

INFORMED CONSENT

Study Title: Effects of Carbohydrate Mouth Rinse Concentration on Cycling Time Trial Performance
Principal Investigator: Jonathan Youell **Co-Investigator:** Dr. Scott Conger
Sponsor: N/A

This consent form will give you the information you will need to understand why this research study is being done and why you are being invited to participate. It will also describe what you will need to do to participate as well as any known risks, inconveniences or discomforts that you may have while participating. We encourage you to ask questions at any time. If you decide to participate, you will be asked to sign this form and it will be a record of your agreement to participate. You will be given a copy of this form to keep.

✎ **PURPOSE AND BACKGROUND**
The purpose of this research study is to determine if rinsing different amounts of sugar in your mouth without swallowing any will improve your exercise performance. This will provide us with information about how sugar affects your body during exercise. To participate in this study, you must be a male between 18 and 45 years of age, in good physical health (no diagnosed cardiovascular, pulmonary, metabolic, joint, or chronic disease), and cycle on average a minimum of 30 miles per week over the least six months.

✎ **PROCEDURES**
You will be asked to come to the Human Performance Laboratory in the Bronco Gym for a total of 5 occasions. Upon completion of all 5 trials, a VO_{2max} test will be provided at no cost to you.

Visit 1 – Paperwork, body composition measurement, and familiarization trial (2 hrs)
During your first visit, you will complete all paperwork, have your body fat measured, and complete a practice time trial. Body fat will be measured with a system that measures the amount of air your body takes up within a chamber. This is an egg-shaped structure that uses changes in air pressure and your body size to determine your total body composition breakdown.

You will be asked to wear a tight fitting bathing suit or lycra/spandex shorts with a swim cap covering the hair to reduce air blockage. You will enter the system and sit for approximately one minute. You will breathe regularly and remain motionless during the testing procedure. A large window is centered in the front of the system so you may see out into the laboratory and may communicate with an investigator if necessary. An emergency release button is located inside the system should you need to terminate the test for any reason.

Next you will perform a practice time trial on a stationary bicycle. This will be identical in nature to the exercise sessions completed during visits 2-5. You will be fitted with a heart rate monitor and asked to urinate approximately 20ml into a cup so that hydration levels can be tested. If you are not well hydrated, we will provide you with water to drink and retest your hydration 15 minutes later. The stationary bicycle will be adjusted so that you can cycle comfortably. You will then complete a 10 minute warm-up in which you will cycle at a self-selected pace and cadence. Following the warm-up, you will complete a 1-hour exercise test where you will ride as far as you can. Every 15 minutes during the exercise session, you will be given a small amount of water (about 2 tablespoons) to swish in your mouth without swallowing for 5 seconds and spit out into a container.
Lastly, you will be given a food and exercise log to complete for three days prior to your next visit. You will be asked to follow a similar diet and exercise regimen before each subsequent visit and to not consume alcohol or caffeine or exercise 24hrs prior to each visit.

Visit 2-5 – Experimental trials (1.5 hrs)
During visits 2-5 you will complete a 10 minute warm up and 1-hour time trial identical to the practice time trial during each visit. Hydration levels will be measured and you will be fitted with a heart rate monitor before beginning. Instead of rinsing with water every 15 minutes, you will rinse with one of 4 randomly assigned drinks containing between 0% and 12% sugar.

✎ **RISKS**
The potential risks that may occur with participating in this study include those associated with any exercise. These include muscle/joint soreness, lightheadedness, nausea, and in rare instances, fainting, and heart attack. However, the possibility of serious events happening in people who have no previous history of heart disease is low. Additionally, there is a risk of emotional stress associated with body composition testing. The Human Performance Laboratory has a planned response to any emergency procedure and all testing personnel are CPR certified.

✎ **BENEFITS**
There will be no direct benefit to you from participating in this study. However, the information that you provide may help scientists to have a greater understanding how the body uses sugar during exercise.

✎ **EXTENT OF CONFIDENTIALITY**
Reasonable efforts will be made to keep the personal information in your research record private and confidential. Any identifiable information obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by law. The members of the research team and the Boise State University Office of Research Compliance (ORC) may access the data. The ORC monitors research studies to protect the rights and welfare of research participants.

Your name will not be used in any written reports or publications which result from this research. Data will be kept for three years (per federal regulations) after the study is complete and then destroyed.

For this research project, the researchers are requesting demographic information. Due to the make-up of Idaho's population, the combined answers to these questions may make an individual person identifiable. The researchers will make every effort to protect your confidentiality. However, if you are uncomfortable answering any of these questions, you may leave them blank.

PAYMENT
You will not be paid for your participation in this study.

PARTICIPATION IS VOLUNTARY
You are free to make a decision to participate in this study, and if you should choose to participate, you may withdraw from the study at any time without penalty. If you withdraw from the study, your data will be given to you or destroyed.

QUESTIONS
If you have any questions or concerns at any time during the course of the study or after completion of the study, you may contact the Principal Investigator, Jonathan Youell at jonathanyouell@u.boisestate.edu.

If you have questions about your rights as a research participant, you may contact the Boise State University Institutional Review Board (IRB), which is concerned with the protection of volunteers in research projects. You may reach the board office between 8:00 AM and 5:00 PM, Monday through Friday, by calling (208) 426-5401 or by writing: Institutional Review Board, Office of Research Compliance, Boise State University, 1910 University Dr., Boise, ID 83725-1138.

DOCUMENTATION OF CONSENT
I have read this form and decided that I will participate in the project described above. Its general purposes, the particulars of involvement and possible risks have been explained to my satisfaction. I understand I can withdraw at any time.

Printed Name of Study Participant	**Signature** of Study Participant	Date

Signature of Person Obtaining Consent	Date

APPENDIX D

Recruitment Flyer

VOLUNTEERS* NEEDED**

For*a*Cycling*Study**

Eligibility:

1. You need to be an 18 to 45 year old male.
2. Cycle at least 30 miles per week for the last 6 months.

What do I need to do?

- Visit one: Body composition testing, familiarization trial
- Visit two-five: 1-hour cycling time trial
- Optional VO2max test provided upon completion

For more information call (253) 777-7997 or send an email to jonathanyouell@u.boisestate.edu

Cycling study
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APPENDIX E

Room Temperature and Barometric Pressure

Table E.1. Room temperature and barometric pressure.

	Placebo	3%	6%	12%
Room Temp (°C)	21.9 ± 0.36	21.3 ± 0.91	21.9 ± 0.5	21.7 ± 0.8
Atmospheric Pressure (mmHg)	692.2 ± 5.0	691.6 ± 4.4	691.5 ± 4.4	691.4 ± 5.5

ANOVA, $p = 0.079$, $p = 0.879$, respectively

APPENDIX F

Participant Body Weight Change and USG

Table F.1. Participant body weight change and USG.

	Placebo	3%	6%	12%
Pre-trial weight (kg)	75.3 ± 10.8	76.2 ± 11.2	75.8 ± 10.9	76.2 ± 11.3
Post-trial weight (kg)	74.4 ± 10.4	74.9 ± 10.8	74.6 ± 10.6	74.8 ± 10.9
Percentage change in body weight	-1.8 ± 0.48	-1.6 ± 0.5	-1.6 ±0.4	-1.7 ± 0.4
USG	1.014 ± 0.007	1.014 ± 0.006	1.008 ± 0.007	1.010 ± .008

ANOVA, $p = 0.108$, $p = 0.707$ respectively.