No dose response effect of carbohydrate mouth rinse on cycling time trial performance

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Running Title: **No dose response effect of carbohydrate mouth rinse**

Abstract

The aim of the present study was to investigate the influence of mouth rinsing carbohydrate at increasing concentrations on ~1 h cycle time trial performance. Eleven male cyclists completed three experimental trials, following an overnight fast. Cyclists performed a ~1 h time trial on a cycle ergometer, while rinsing their mouth for 5 s with either a 7% maltodextrin solution (CHO), 14% CHO or a taste-matched placebo (PLA) after every 12.5% of the set amount of work. Heart rate was recorded every 12.5% of the time trial, whilst RPE and GI comfort were determined every 25% of the time trial. The mouth rinse protocol influenced the time to complete the time trial ($P<0.001$), with cyclists completing the time trial faster during 7% CHO (57.3 ± 4.5 min; $P=0.004$) and 14% CHO (57.4 ± 4.1 min; $P=0.007$), compared to PLA (59.5 ± 4.9 min). There was no difference between the two carbohydrate trials ($P=0.737$). There was a main effect of time ($P<0.001$) for both heart rate and RPE, but no main effect of trial ($P=0.107$ and $P=0.849$, respectively). Scores for GI comfort ranged from 0-2 during trials, indicating very little GI discomfort during exercise. In conclusion, mouth rinsing and expectorating a 7% maltodextrin solution, for 5 s routinely during exercise was associated with improved cycle time trial performance approximately 1 h in duration. Increasing the carbohydrate concentration of the rinsed solution from 7% to 14% resulted in no further performance improvement.

Word count: 240

Key Words: Maltodextrin; Endurance exercise performance; Oral cavity.

Introduction

The ingestion of carbohydrate during prolonged exercise has been reported to delay the onset of fatigue and enhance endurance capacity (Coggan & Coyle, 1987; Tsintzas & Williams, 1998). Carbohydrate exerts its effect by maintaining blood glucose concentrations and providing an exogenous substrate for metabolism in the later stages of exercise (Coyle et al., 1986; Jeukendrup, 2004; Neufer et al., 1987). Furthermore, carbohydrate ingestion may result in a more gradual depletion of endogenous glycogen stores (Tsintzas et al., 1996). However, improvements in endurance capacity have also been reported without evidence of glycogen sparing (Coyle et al., 1986).

During shorter duration exercise (≤1 h), endogenous stores of carbohydrate are unlikely to be limiting. Therefore, there is no clear metabolic rationale for ingesting carbohydrate. Nevertheless, some studies (Below et al., 1995; Carter et al., 2003; Jeukendrup et al., 1997; Neufer et al., 1987; Rollo & Williams 2009) but not all (Anantaraman et al., 1995; Desbrow et al., 2004; Widrick et al., 1993) have shown a performance benefit of ingesting carbohydrate during short-duration, high-intensity exercise such as time trials of ≤1 h duration.

Since the first study by Carter et al. (2004), several studies have shown that mouth rinsing a carbohydrate solution without ingestion is associated with similar improvements in self-selected endurance (~1 h) performance as observed when carbohydrate is ingested (Chambers et al., 2009; Lane et al., 2013; Rollo et al., 2010). The mechanism(s) by which mouth rinsing with a carbohydrate solution influences self-selected power output and thus endurance performance are unknown. The expectoration of carbohydrate solution prevents substrate delivery to the systemic circulation, and as such it has been speculated that carbohydrate recognition in the oral cavity evokes a central effect during exercise (Jeukendrup et al., 2013;

Rollo & Williams, 2011). The first study to draw the association between a central response and exercise performance was completed by Chambers et al. (2009). The authors reported that mouth rinsing with both a sweet and a non-sweet carbohydrate solution (6.4% glucose and maltodextrin, respectively) was associated with improved 1 h cycling time trial performance. In addition, mouth rinsing with an 18% maltodextrin solution was reported to activate regions of the brain associated with reward (Chambers et al., 2009; Rolls, 2007). Interestingly, the activation of reward centres in the brain have been reported to be sensitive to the calorific value of the maltodextrin ingested (Smeets et al., 2005; van Rijn et al., 2015). Thus, if the concentration of carbohydrate rinsed in the mouth activates a central reward response in a dose-dependent manner, there may be a subsequent dose-response associated with improvements in exercise performance.

To date, three studies have investigated the dose-response relationship between carbohydrate concentration and endurance performance. The first reported that 90 min running performance was improved with a 6% carbohydrate-electrolyte solution compared to a placebo with no further improvement when rinsing with a 12% solution (Wright & Davison, 2013). More recently, two studies have reported that increasing the concentration of maltodextrin in the rinsed solution has no effect on endurance cycling performance. Specifically, Ispoglou et al. (2015) reported that when seven trained male cyclists rinsed with 0, 4, 6, and 8 % carbohydrate solutions, there were no performance differences between any trials for a 1 h time trial performance. Similar findings were reported when nine recreationally active males mouth rinsed with a 0, 3, 6 and 12 % carbohydrate solutions during a 20 km time trial (Kulaksiz et al., 2016). However, the use of untrained/ inexperienced cyclists (Kulaksiz et al., 2016; Wright & Davison, 2013), extremely large performance improvements (up to 18.6 % improvement between trials; Wright & Davison,

2013) and short periods of fasting prior to the exercise test (only 3 h post prandial; Ispoglou et al., 2015) are all limitations in study design for these investigations.

Therefore, the purpose of the present study was to investigate if a dose response relationship exists between the concentration of a carbohydrate mouth rinse solution and endurance cycling performance, in endurance trained cyclists. Our hypothesis was that greater carbohydrate concentrations in the rinsed solution would be associated with greater improvements in cycle time trial performance.

Methods

Subjects

After institutional ethical approval, 12 competitive male cyclists completed a health screen questionnaire and provided written consent, but the data from one subject was omitted as it later transpired he had not adequately controlled physical activity before trials. All subjects were cyclists accustomed to training and/or competitions lasting at least 1 hour. The physical characteristics (mean ± SD) of the subjects were age: 40 ± 8 years; weight: 77.6 ± 7 kg; height: 1.79 ± 0.07 m; $\dot{V}O_{2peak}$: 58 ± 11 $ml \cdot kg^{-1} \cdot min^{-1}$.

Experimental Design

Subjects completed two preliminary trials, followed by three experimental trials that were administered in a randomised, double blinded study design. In all trials, exercise was completed on the same electrically braked cycle ergometer (Lode Excalibur, Ggroningen, Netherlands).

Preliminary sessions

110 During the first visit, peak oxygen uptake ($\dot{V}O_{2peak}$) and peak power output (W_{peak}) were
111 determined using an incremental exercise test. Workload was initially set at 95 W, and
112 increased by 35 W every 3 min, until exhaustion. One minute expired air samples were
113 collected into a Douglas bag at the end of each stage and at exhaustion. The preferred seat
114 height and handle bar position for each subject was noted and was repeated in subsequent
115 visits. During the second preliminary session, subjects completed the full time trial used in
116 the experimental trials to habituate them to the protocol. During the familiarisation trial,
117 subjects rinsed their mouth with the placebo solution used in the experimental trials.

118 *Experimental trials*

119 Experimental trials took place in the morning following an overnight fast at a time
120 standardised for each subject. Trials were separated by at least one week. On the day
121 preceding the first experimental trial, subjects recorded their dietary intake and any habitual
122 low intensity physical activity in a diary, replicating these patterns of diet and activity before
123 subsequent trials. Adherence to this was checked verbally before each trial. During this time,
124 subjects abstained from alcohol intake and any strenuous exercise.

125 Upon arrival at the laboratory, subjects provided a urine sample, which was analysed for
126 osmolality using a handheld refractometer (Atago PAL-1, Japan) and attached a heart rate
127 monitor (Polar, Kempele, Finland). Following a brief warm-up (5 minutes at 40% W_{peak} , 5
128 minutes at 60% W_{peak} and 3 minutes of self-selected stretching), subjects completed a
129 simulated cycling time trial, during which they were required to complete a set amount of
130 work (844 ± 63 kJ) as fast as possible. The total amount of work for completion was
131 standardised for each subject and was equivalent to cycling for 1 hour at 75% W_{peak}. This was
132 calculated according to the following formula (Carter et al., 2004):

133 Total work = 0.75 x W_{peak} x 3600 s

The ergometer was set in linear mode so that 75% W_{max} was obtained when pedalling at the subject's preferred cadence, determined during the VO_{2peak} test. Subjects received no performance-related information (exercise time, heart rate or cadence) other than the accumulated work performed displayed on a computer screen and no encouragement was provided to subjects during trials. At the start and every 12.5% of the time trial thereafter, subjects rinsed and expectorated 25 ml of one of the three solutions. Solutions were a carbohydrate-free placebo solution (PLA) and two carbohydrate solutions made up using maltodextrin to provide a final weight/volume concentration of 7% (7% CHO) or 14% (14% CHO) maltodextrin. Solutions were taste-matched and made up using 200 ml/l single concentrate no-added sugar orange and pineapple flavour squash (Robinsons Soft Drinks Ltd, UK). Each 25 ml was delivered via a plastic syringe and subjects rinsed the solution around their mouth for 5 seconds before expectorating into a pre-weighed plastic container. The syringe and plastic container were weighed before and after each mouth rinse using an electronic balance (Argos, Stafford, UK) to determine the volume of fluid rinsed and expectorated, in order to determine whether any fluid was unintentionally ingested. The temperature of the rinse solution was measured at the start of each trial using a mercury in glass thermometer. Heart rate was recorded every 12.5% of the time trial, whilst RPE and GI comfort were determined every 25% of the time trial. RPE was determined using the 6 to 20 point Borg scale (Borg, 1982), and GI comfort was assessed using a 12-point scale, with anchors provided at 0 ”neutral”, 4 ”uncomfortable”, 8 ”very uncomfortable” and 12 ”painful”. Time to complete each 12.5%, as well as time to complete the entire time trial was recorded.

On completion of the final trial, subjects were asked if they had been able to distinguish between the solutions rinsed during each trial; if so, they were asked to identify which solution they thought was which.

Statistical Analyses

Data are reported as mean and standard deviation (mean ± SD), unless otherwise stated. All data were analysed using SPSS software package (version 21.0; SPSS Inc, Chicago, IL, USA). A Sharipo-Wilk test was used to test for normality of distribution. Overall time trial performance, trial order effect, body mass, urine osmolality, environmental conditions and solution temperature and expectorated volume were all analysed using a one way repeated measures analysis of variance (ANOVA). A two-way repeated measures ANOVA (trial x time) was used to examine performance for each 12.5% of the time trial, heart rate, RPE and GI comfort. Post-hoc paired t-tests or Wilcoxon Signed Rank tests were used as appropriate and the Holm-Bonferroni adjustment was used to control the family-wise error rate. Statistical significance was accepted when $P<0.05$.

Results

Time trial

There was no trial order effect for time to complete the time trial, with performance times of 58.1 ± 4.5 min, 57.8 ± 4.4 min and 58.2 ± 5.0 min on the first, second and third trials, respectively ($P=0.761$). The mouth rinse protocol influenced the time to complete the time trial (Figure 1; $P<0.001$), with subjects completing the time trial faster during 7% CHO (57.3 ± 4.5 min; $P=0.004$) and 14% CHO (57.4 ± 4.1 min; $P=0.007$), compared to PLA (59.5 ± 4.9 min), with no difference between the two CHO trials ($P=0.737$). Whilst there were main effects of time ($P<0.001$) and trial ($P<0.001$) for time to complete each 12.5% of the time trial, there was no interaction effect ($P=0.221$), indicating similar pacing between trials (Figure 2). There was no difference between trials for environmental temperature ($P=0.550$)

or relative humidity (P=0.345), and across all trials these variables were 21.6 ± 1.1°C and 50.3 ± 4.4%, respectively.

Pre-trial measures

There was no difference for pre-trial body mass (PLA: 78.6 ± 6.2 kg; 7% CHO: 78.6 ± 6.4 kg; 14% CHO: 78.7 ± 6.2 kg; P=0.783), urine osmolality (PLA: 339 ± 187 mOsm·kg^{-1}; 7% CHO: 329 ± 186 mOsm·kg^{-1}; 14% CHO: 365 ± 206 mOsm·kg^{-1}; P=0.788)) or resting heart rate (PLA: 67 ± 7 beat·min^{-1}; 7% CHO: 66 ± 7 beat·min^{-1}; 14% CHO: 66 ± 6 beat·min^{-1}; P=0.830).

Heart rate, RPE and GI comfort

There was a main effect of time (P<0.001), but no main trial (P=0.107) or interaction effect (P=0.391) for heart rate (Table 1). There was also a main effect of time (P<0.001) but no main trial (P=0.849) or interaction effect (P=0.787) for RPE (Table 1). There was no time (P=0.123), trial (P=0.422) or interaction (P=0.864) effect for GI comfort. Scores for GI comfort ranged from 0-2 during trials, indicating very little GI discomfort was present during exercise (Table 1).

Rinse solution temperature, expectorate volume and solution detection

There was no difference between trials in the temperature of the rinse solution (PLA: 13.4 ± 4.2 °C; 7%: 12.2 ± 2.3 °C; 14%: 13.7 ± 2.8 °C; P=0.625) or the volume of rinse solution expectorated (PLA: 24.5 ± 1.1 ml; 7%: 24.9 ± 1.4 ml; 14%: 24.9 ± 1.3 ml; P=0.627). Seven of the eleven subjects failed to distinguish between the rinse solutions. The remaining four correctly differentiated the placebo from the two carbohydrate solutions, but only one correctly distinguished between the 7% and 14% concentrations.

Discussion

The main finding of this study was that no further improvement in ~1h cycle time trial performance was observed when the carbohydrate concentration of the rinsed solution was increased from 7% to 14%, compared to a taste matched placebo. Thus, we reject our hypothesis that there would be a dose response effect of carbohydrate concentration on endurance performance.

The findings of this study support those of Wright and Davison (2013), who showed that there was no additional performance benefit of mouth rinsing a 12% carbohydrate solution over that observed between a 6% solution and a placebo. Wright and Davison (2013) recruited 7 males who were instructed to cover as much distance as possible in a 90 min treadmill test, rinsing their mouth at 0, 15, 30 and 45 min of the protocol. However, the participants only covered relatively short distances (Placebo 13.9 ± 1.7 km; 6% CHO 14.6 ± 1.7 km; 12% CHO 14.9 ± 1.6 km), suggesting the population were not well trained, despite being reported to be in competitive sports teams. Furthermore, extremely large performance improvements seen in some trials (up to 18.6%) far exceed the typical improvements seen in performance studies, calling into question either the standardisation of pre-trial conditions or the variability of the protocol employed. The present study used the same cycling time trial protocol as the original mouth rinse studies (Carter et al., 2004; Chambers et al., 2009), which has a reported variability of 3.35 % in trained cyclists (Jeukendrup et al., 1996). As such, we have confidence that the observed differences between performance trials in the present study were a consequence of the carbohydrate rinse intervention.

In contrast to the present study and that of Wright and Davison (2013), two other dose-response studies have reported no effect of carbohydrate mouth rinse on endurance performance. Ispoglou et al. (2015) used the same performance time trial and rinse regimen

as the present study and showed no effect of mouth rinsing with 4, 6, or 8% carbohydrate (89% sucrose; 11% glucose) solutions compared to a 0% placebo. However, the cyclists had ingested a meal 3 h prior to exercise and were therefore not in a fasted state during the trials (Ispoglou et al., 2015). Although Lane et al. (2013) reported that mouth rinsing a 10% maltodextrin solution for 10 s improved 60 min cycle time trial performance in both fed and fasted conditions, the magnitude of improvement was greater in the fasted condition. Furthermore, Beelen and collegues (2009) have shown that 1 h cycling time trial performance is not influenced by mouth rinsing a 6.4% maltodextrin solution compared to water when cyclists ingest ~2.5 g carbohydrate·kgBM^{-1} two hours before the test. Indeed, imaging studies have shown that the central activation of reward centres in the brain in response to carbohydrate feedings are diminished under conditions of satiety in comparison to hunger (Haase et al., 2009). Thus, although providing a carbohydrate rich meal prior to exercise may have some ecological validity, it is not favourable to detecting small performance benefits that carbohydrate mouth rinse may provide (Rollo et al., 2010).

More recently Kulaksiz et al. (2016) reported that 20 km cycle time trial performance was not influenced by mouth rinsing either 3%, 6% or 12% maltodextrin solutions compared to a 0% placebo. Direct comparisons to the present study are difficult due to differences in protocol used and training status of the participants. Kulaksiz et al (2016) recognised that the $\dot{V}O_2$max values of their participants were lower (~21-42%) than those recruited to previous mouth rinse studies (Carter et al., 2004; Chambers et al., 2009; Lane et al., 2013). Although Kulaksiz et al. (2016) used a validated protocol (Zavorsky et al., 2007), it has been shown that top performers (i.e., those cyclists that maintained a higher average power output over 20 km) had a coefficient of variation that was four times lower compared to the bottom performers (1.2% and 4.8 %, respectively; Zavorsky et al., 2007). The mean power output in the study by Kulaksiz et al. (2016) was lower (~200 Watts) than the bottom cyclists in the

validation study (~260 Watts), suggesting that the population recruited may not have been appropriate for the test used.

A limitation of the present study was that a no-rinse control trial was not included in the study design and Gam et al. (2013) have suggested that mouth rinsing *per se* during exercise maybe detrimental to performance (Gam et al., 2013). Nevertheless, the results of the present study are consistent with previous cycling studies reporting that routinely mouth rinsing and expectorating a carbohydrate solution during exercise increases self-selected power outputs during cycling time trials of approximately 1 h in duration (Carter et al., 2004; Chambers et al., 2009; Lane et al., 2013; Pottier et al., 2008). Indeed, Pottier et al. (2008) showed that mouth rinsing and expectorating a carbohydrate solution had a greater performance benefit compared to ingesting (14 ml·kgBM·h^{-1}) the same solution without rinsing (3.7% vs 1.4%, respectively). Despite the oral cavity being exposed to carbohydrate in both trials, the discrepancy in performance was attributed to the short oral transit time when the carbohydrate-electrolyte solution was ingested (Pottier et al., 2008). To support this hypothesis, Sinclair et al. (2014) reported that 30 min cycle time trial performance was improved by doubling the duration (5 s to 10 s) that a 6.4% maltodextrin solution was rinsed in the mouth. Whether an increased duration of rinse would have influenced the results in the present study is unknown, however prolonged rinsing may interfere with participants breathing patterns during high intensity exercise and therefore potentially become a confounding factor (Gam et al., 2013). Regardless, while there may be a dose response when doubling the duration of carbohydrate exposure to the oral cavity (Sinclair et al., 2014), the results of the present study suggest that this dose response does not extend to doubling the concentration of carbohydrate in the rinsed solution (Figure 1).

The mechanism(s) by which endurance performance is improved by mouth rinsing and expectorating carbohydrate solutions remain unknown. Previous studies have speculated that

the presence of carbohydrate exerts a central response during exercise and manifests as improved performance (Carter et al., 2004; Chambers et al., 2009). Observations from imaging studies at rest have reported that regions in the brain, specifically the insula/frontal operculum, oribitofrontal cortex and striatum, are activated when carbohydrate enters the oral cavity, independent of sweetness (Chambers et al., 2009). These regions of the brain activated by carbohydrate in the oral cavity are believed to be associated with reward and sensory perception (Turner et al., 2014) which may influence behavioural responses (Kringelbach et al., 2004). Receptors (T1R2 and T1R3) within the mouth are likely to signal that carbohydrates are rewarding due to both palatability and caloric value (Berthoud 2003; Smeets et al., 2005; van Rijn et al., 2015). Thus, speculatively, mouth rinsing a carbohydrate solution provides the promise of exogenous energy to the brain when liver and muscle glycogen stores are depleted. However, increasing the energy content of the carbohydrate rinse solution that the oral cavity is exposed to (i.e., from 7% to 14% in the present study) had no measurable impact on performance or perception of effort (Figure 1, Table 1).

Carbohydrate mouth rinse has been reported to increase the activation of cortico-motor pathways and voluntary force production in both fresh and fatigued muscle involved in elbow flection (Gant et al., 2010). Consistent with endurance performance studies, the neuromuscular response to mouth rinsing carbohydrate has been reported to be more sensitive when participants have lower endogenous carbohydrate stores (Ataide-Silva et al., 2016). Furthermore, mouth rinsing a 6.4% maltodextrin solution was shown to maintain electromyographic activity and enhance whole body, moderate intensity exercise performance (Bastos-Silva et al., 2016). To this end, the mechanism by which carbohydrate mouth rinse influences exercise performance may not be solely a consequence of promised exogenous energy delivery to the brain, but may also be directly evoking central motor responses.

In conclusion, mouth rinsing and expectorating a 7% maltodextrin solution, for 5s routinely during exercise was associated with improved ~1h cycling time trial performance. No dose response relationship was observed. Therefore, the practical implications of this study suggest that, under fasting conditions, mouth rinsing a 7% carbohydrate solution may offer a performance benefit to athletes in cycling time trial performances of approximately 1h. There is no further benefit from rinsing a more concentrated carbohydrate solution.

Authorships, declarations of funding sources and conflicts of interest,

The study was designed by RMJ and LJJ; data were collected and analysed by RMJ and SR; data interpretation and manuscript preparation were undertaken by RMJ, IR and LJJ. All authors approved the final version of the paper. No funding was received for this work. IR is an employee of the Gatorade Sports Science Institute, a division of PepsiCo Inc. The views expressed in this manuscript are those of the authors and do not necessarily reflect the position or policy of PepsiCo Inc. All other authors have no conflict of interest to declare.

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417 **Tables**

418 Table 1. Heart rate (beats·min^{-1}), rating of perceived exertion (6-20) and gastrointestinal
419 comfort (0-12) every 25% of time trial. Data are expressed as mean ± SD.

	25%	50%	75%	100%
Heart rate (beats·min^{-1})				
PLA	139 ± 14	144 ±15	147 ± 18	157 ± 18
7% CHO	140 ± 15	146 ± 16	148 ± 16	159 ± 17
14% CHO	136 ± 14	141 ± 16	146 ± 17	157 ± 18
RPE (6-20)				
PLA	14 ± 2	16 ± 1	16 ± 2	18 ± 2
7% CHO	13 ± 2	15 ± 1	16 ± 1	18 ± 2
14% CHO	14 ± 1	16 ± 1	16 ± 2	18 ± 2
Gastrointestinal comfort (0-12)				
PLA	0 ± 0	0 ± 1	1 ± 1	1 ± 1
7% CHO	0 ± 1	0 ± 1	1 ± 1	1 ± 1
14% CHO	1 ± 1	1 ± 1	1 ± 1	1 ± 1

420

421

422 **Figure Legends**

423 Figure 1. Time to complete the time trial during PLA, 7% CHO and 14% CHO. Top panel
424 displays mean ± SD values. Bottom panel displays individual subject data. # denotes a
425 significant difference from PLA trial.

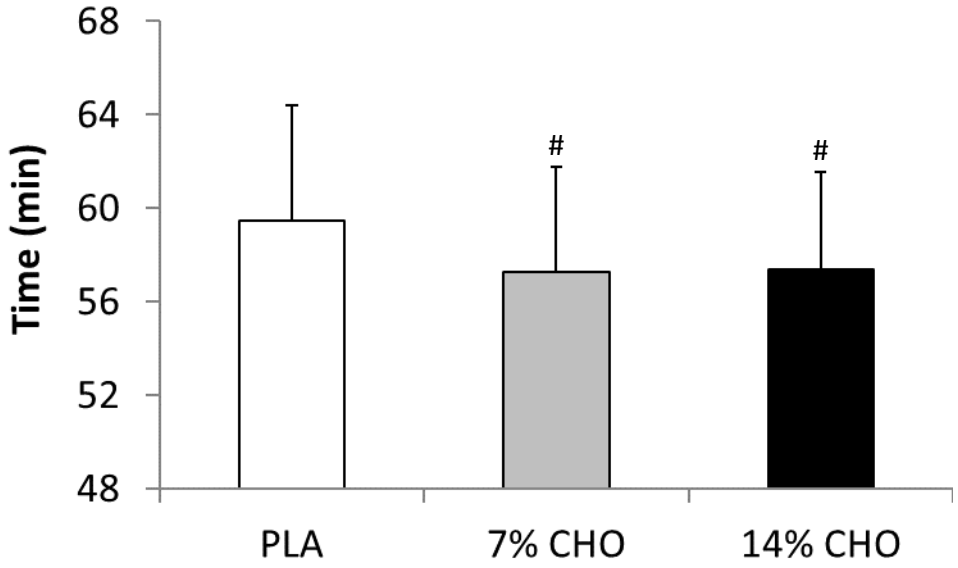
426

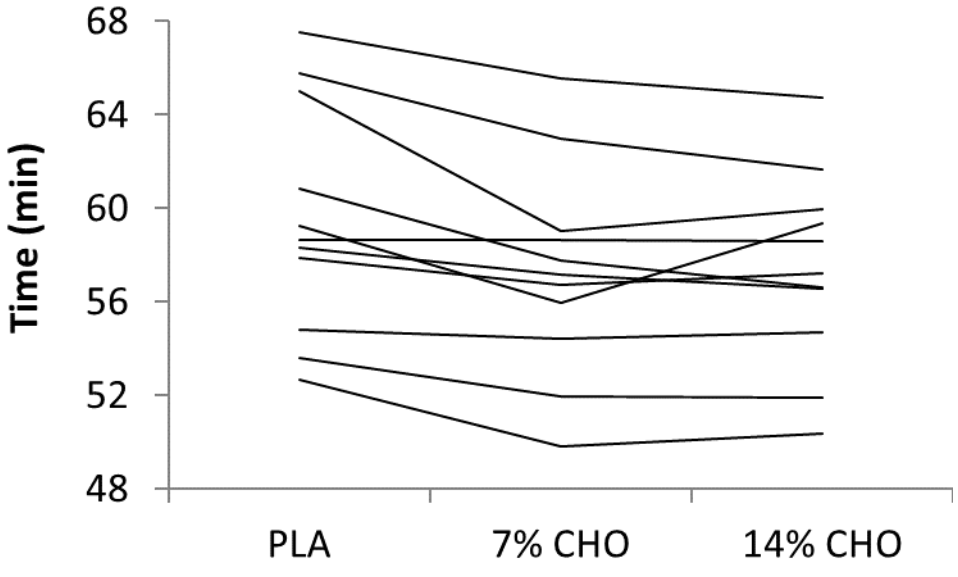
427 Figure 2. Time to complete each 12.5% segment of the time trial in the PLA, 7% CHO and
428 14% CHO trials. Data are expressed as mean ± SD. There was a main effect of time
429 ($P<0.001$) and trial ($P<0.001$), but no interaction effect.

430

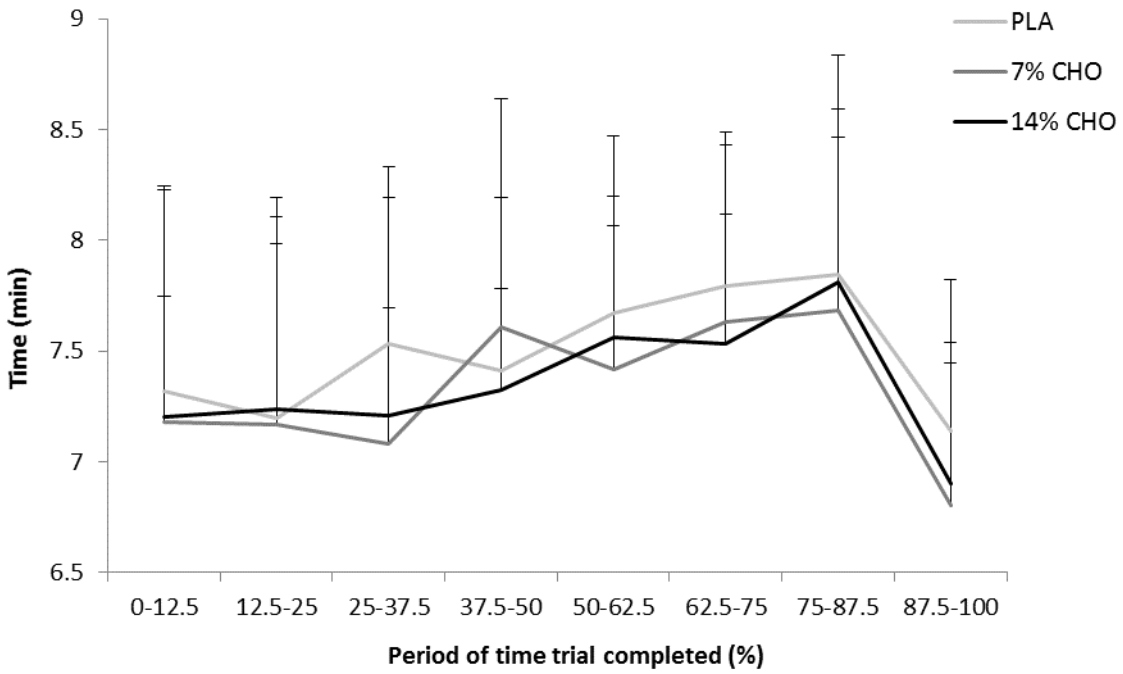
Figures

Figure 1.





435 Figure 2.



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