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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.

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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

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

Experimental trials

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

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

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

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

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

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

310

311 **Authorships, declarations of funding sources and conflicts of interest,**

312 The study was designed by RMJ and LJJ; data were collected and analysed by RMJ and SR;
313 data interpretation and manuscript preparation were undertaken by RMJ, IR and LJJ. All
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315 an employee of the Gatorade Sports Science Institute, a division of PepsiCo Inc. The views
316 expressed in this manuscript are those of the authors and do not necessarily reflect the
317 position or policy of PepsiCo Inc. All other authors have no conflict of interest to declare.

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Tables

Table 1. Heart rate (beats·min^{-1}), rating of perceived exertion (6-20) and gastrointestinal 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

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.

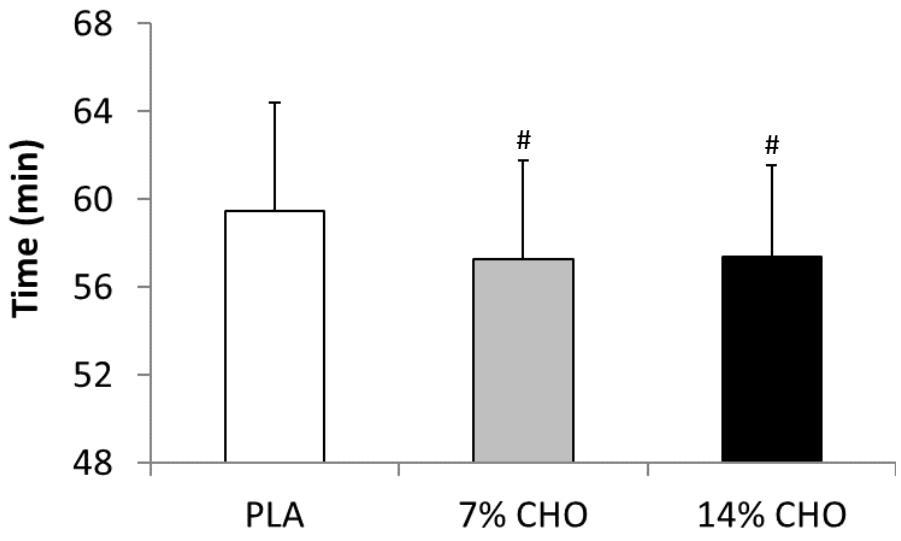
426

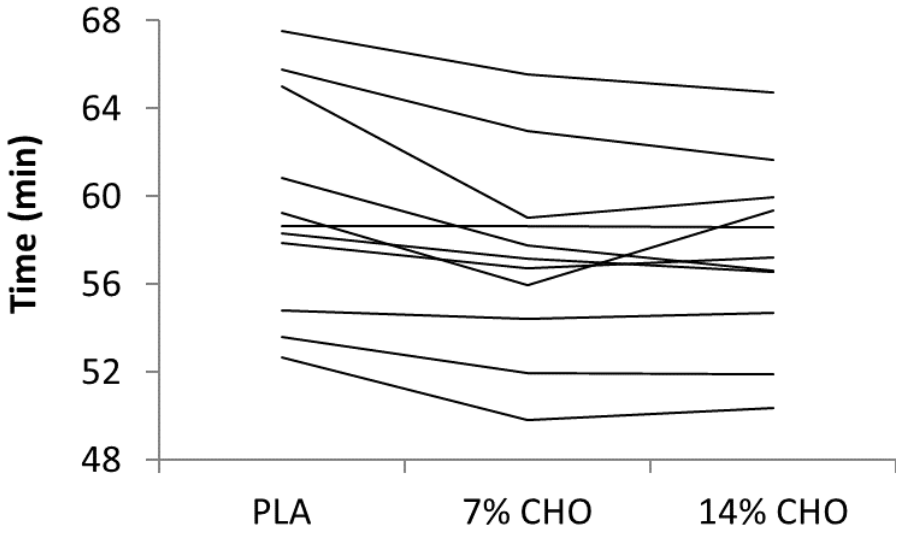
427 Figure 2. Time to complete each 12.5% segment of the time trial in the PLA, 7% CHO and
428 14% CHO trials. Data are expressed as mean ± SD. There was a main effect of time
429 ($P<0.001$) and trial ($P<0.001$), but no interaction effect.

430

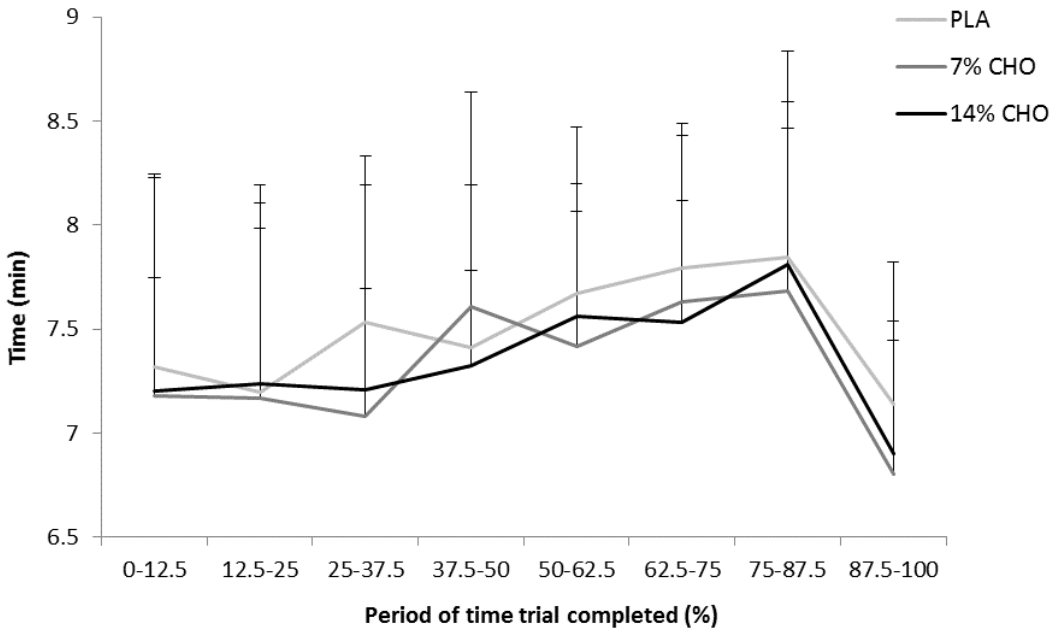
Figures

Figure 1.





435 Figure 2.



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Reviewer(s)' Comments to Author – Round 2

Reviewer: 1

Recommendation: Minor Revision

Comments:
The manuscript is much improved with the changes to the introduction and discussion sections. *Thank you for taking the time to re-review our manuscript.* I am satisfied that my major concerns have been addressed, I just have some minor comments remaining.

Minor Comments:

Regarding the response to my previous comment on whether pre-trial nutritional intake was quantified, I agree that the standardization of diet and activity patterns as described in the manuscript is sufficient for this type of study. It is, however, unfortunate that compliance was only verbally confirmed by the participants and that food/activity diaries from the 24 hours preceding each trial were not reviewed by the investigators as this would have provided an added level of rigor. *Yes, we agree and we will certainly look to do this in future.*

Page 4 line 66 - Please specify the composition of the sweet CHO solution in the Chambers study. *The sweet CHO was achieved with glucose. The sentence has now been changed to read:* 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.

Page 4 line 79 - "that that". Please correct. *This has been corrected.*

Page 10 line 226 - "Wright And Davison". Change to "and". *This has been corrected.*

Page 11 line 244 - What were the CHO solutions composed of in the Kulaksiz study? *They used maltodextrin, and we have now changed the word carbohydrate to maltodextrin to clarify this.*

Page 12 line 273 - Correct the reference for "Gam et al., 2010" to "2013". *This has been corrected.*

Page 13 line 282 - "cortext". Please correct this spelling. *This has been corrected.*

Page 13 line 290 - I don't think the term "lowered by exercise" is appropriate here. Are there any studies which have tested the effect of CHO mouth rinsing after purposefully depleting endogenous glycogen stores through exercise? To my knowledge, most studies have been performed in a state of reduced exogenous CHO availability induced by fasting. *To our knowledge studies have indeed used fasting rather than exercise to deplete endogenous glycogen. We have therefore altered this sentence to read simply :* Thus, speculatively, mouth rinsing a carbohydrate solution provides the promise of exogenous energy to the brain when liver and muscle glycogen stores are depleted.

Reviewer: 2

Recommendation: Minor Revision

Comments:
Dear Author,

Good job on the revised manuscript. *Thank you for taking the time to re-review our manuscript*. It has addressed majority of the concerns raised, however, there are two issues which I feel were only partially addressed. The following are my comments describing these issues.

Comments:

One of the aims of this study was to address the research design limitations (ie training status of participants and short periods of fasting prior to exercise test) of other studies that investigated the dose-response relationship between CHO concentration and endurance performance.

1. In order to address the limitation of training status of the participants, the author recruited cyclists that were accustomed to training and/or competitions lasting at least 1 h. Although the author cited a few studies to justify the variability of the cycling time trial protocol and training status of the participants used in this study, the typical error for the 1h time trial performance with their participants was not established. Due to the lack of data in the typical error in performance, it is a pity that the study cannot fully address the training status limitation of other studies. Nevertheless, I acknowledge that it is always a challenge for practitioners to get trained participants/athletes to perform two additional testing sessions to establish their typical error without disputing their training programme. *Wherever possible we do try to ensure that we can report the variability of our measurements if required, but alas the use of subjects with job, family and training demands on their time means that additional testing sessions were not possible. We chose a robust performance test as the compromise between the perfect study design and the achievable study design.*

2. In this present study, the author highlighted that the participants in the study of Ispoglou et al. (2015) were required to fast for ~3h prior to their 1h cycling time trial and such short period of fasting was viewed as a research study limitation. Although the nutritional status and length of fasting may influence the efficacy of carbohydrate mouth rinse, the short fasting period in the study of Ispoglou et al. (2015) shouldn't be considered a limitation, but an ecological valid research design that mimics real world practises, even more so with high intensity events that last more than an hour. Thus, the point on the fasting period does not provide a strong and valid justification for this study. *Thank you for this comment. We agree regarding your point about the ecological validity of the Ispoglou study and have therefore altered the concluding sentence of the paragraph (L226-242) addressing this subject to read :* 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.

Reviewer: 3

Recommendation: Accept

Comments:
The authors have adequately altered/answered all my suggestions/questions. I have no reservation to recommend this paper for publication in this current form.
Thank you for taking the time to re-review our manuscript.