



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

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| Journal: | <i>International Journal of Sport Nutrition & Exercise Metabolism</i> |
| Manuscript ID | IJSNEM.2016-0111.R2 |
| Manuscript Type: | Original Research |
| Keywords: | Maltodextrin; Endurance exercise performance; Oral cavity. |
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Manuscripts

Peer Review

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

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

18 Abstract

19 The aim of the present study was to investigate the influence of mouth rinsing carbohydrate
20 at increasing concentrations on ~1 h cycle time trial performance. Eleven male cyclists
21 completed three experimental trials, following an overnight fast. Cyclists performed a ~1 h
22 time trial on a cycle ergometer, while rinsing their mouth for 5 s with either a 7%
23 maltodextrin solution (CHO), 14% CHO or a taste-matched placebo (PLA) after every 12.5%
24 of the set amount of work. Heart rate was recorded every 12.5% of the time trial, whilst RPE
25 and GI comfort were determined every 25% of the time trial. The mouth rinse protocol
26 influenced the time to complete the time trial ($P < 0.001$), with cyclists completing the time
27 trial faster during 7% CHO (57.3 ± 4.5 min; $P = 0.004$) and 14% CHO (57.4 ± 4.1 min;
28 $P = 0.007$), compared to PLA (59.5 ± 4.9 min). There was no difference between the two
29 carbohydrate trials ($P = 0.737$). There was a main effect of time ($P < 0.001$) for both heart rate
30 and RPE, but no main effect of trial ($P = 0.107$ and $P = 0.849$, respectively). Scores for GI
31 comfort ranged from 0-2 during trials, indicating very little GI discomfort during exercise. In
32 conclusion, mouth rinsing and expectorating a 7% maltodextrin solution, for 5 s routinely
33 during exercise was associated with improved cycle time trial performance approximately 1 h
34 in duration. Increasing the carbohydrate concentration of the rinsed solution from 7% to 14%
35 resulted in no further performance improvement.

36

37 **Word count:** 240

38

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

40 Introduction

41 The ingestion of carbohydrate during prolonged exercise has been reported to delay the onset
42 of fatigue and enhance endurance capacity (Coggan & Coyle, 1987; Tsintzas & Williams,
43 1998). Carbohydrate exerts its effect by maintaining blood glucose concentrations and
44 providing an exogenous substrate for metabolism in the later stages of exercise (Coyle et al.,
45 1986; Jeukendrup, 2004; Neuffer et al., 1987). Furthermore, carbohydrate ingestion may result
46 in a more gradual depletion of endogenous glycogen stores (Tsintzas et al., 1996). However,
47 improvements in endurance capacity have also been reported without evidence of glycogen
48 sparing (Coyle et al., 1986).

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

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

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

75 To date, three studies have investigated the dose-response relationship between carbohydrate
76 concentration and endurance performance. The first reported that 90 min running
77 performance was improved with a 6% carbohydrate-electrolyte solution compared to a
78 placebo with no further improvement when rinsing with a 12% solution (Wright & Davison,
79 2013). More recently, two studies have reported that increasing the concentration of
80 maltodextrin in the rinsed solution has no effect on endurance cycling performance.
81 Specifically, Ispoglou et al. (2015) reported that when seven trained male cyclists rinsed with
82 0, 4, 6, and 8 % carbohydrate solutions, there were no performance differences between any
83 trials for a 1 h time trial performance. Similar findings were reported when nine
84 recreationally active males mouth rinsed with a 0, 3, 6 and 12 % carbohydrate solutions
85 during a 20 km time trial (Kulaksiz et al., 2016). However, the use of untrained/
86 inexperienced cyclists (Kulaksiz et al., 2016; Wright & Davison, 2013), extremely large
87 performance improvements (up to 18.6 % improvement between trials; Wright & Davison,

88 2013) and short periods of fasting prior to the exercise test (only 3 h post prandial; Ispoglou
89 et al., 2015) are all limitations in study design for these investigations.

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

95

96 **Methods**

97 *Subjects*

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

104 *Experimental Design*

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

109 *Preliminary sessions*

110 During the first visit, peak oxygen uptake ($\dot{V}O_{2\text{peak}}$) 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 = $0.75 \times W_{\text{peak}} \times 3600$ s

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

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

159 *Statistical Analyses*

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

170

171 **Results**172 *Time trial*

173 There was no trial order effect for time to complete the time trial, with performance times of
174 58.1 ± 4.5 min, 57.8 ± 4.4 min and 58.2 ± 5.0 min on the first, second and third trials,
175 respectively ($P=0.761$). The mouth rinse protocol influenced the time to complete the time
176 trial (Figure 1; $P < 0.001$), with subjects completing the time trial faster during 7% CHO (57.3
177 ± 4.5 min; $P=0.004$) and 14% CHO (57.4 ± 4.1 min; $P=0.007$), compared to PLA (59.5 ± 4.9
178 min), with no difference between the two CHO trials ($P=0.737$). Whilst there were main
179 effects of time ($P < 0.001$) and trial ($P < 0.001$) for time to complete each 12.5% of the time
180 trial, there was no interaction effect ($P=0.221$), indicating similar pacing between trials
181 (Figure 2). There was no difference between trials for environmental temperature ($P=0.550$)

182 or relative humidity ($P=0.345$), and across all trials these variables were $21.6 \pm 1.1^{\circ}\text{C}$ and
183 $50.3 \pm 4.4\%$, respectively.

184 *Pre-trial measures*

185 There was no difference for pre-trial body mass (PLA: 78.6 ± 6.2 kg; 7% CHO: 78.6 ± 6.4
186 kg; 14% CHO: 78.7 ± 6.2 kg; $P=0.783$), urine osmolality (PLA: 339 ± 187 mOsm \cdot kg $^{-1}$; 7%
187 CHO: 329 ± 186 mOsm \cdot kg $^{-1}$; 14% CHO: 365 ± 206 mOsm \cdot kg $^{-1}$; $P=0.788$) or resting heart
188 rate (PLA: 67 ± 7 beat \cdot min $^{-1}$; 7% CHO: 66 ± 7 beat \cdot min $^{-1}$; 14% CHO: 66 ± 6 beat \cdot min $^{-1}$;
189 $P=0.830$).

190 *Heart rate, RPE and GI comfort*

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

197 *Rinse solution temperature, expectorate volume and solution detection*

198 There was no difference between trials in the temperature of the rinse solution (PLA: $13.4 \pm$
199 4.2 $^{\circ}\text{C}$; 7%: 12.2 ± 2.3 $^{\circ}\text{C}$; 14%: 13.7 ± 2.8 $^{\circ}\text{C}$; $P=0.625$) or the volume of rinse solution
200 expectorated (PLA: 24.5 ± 1.1 ml; 7%: 24.9 ± 1.4 ml; 14%: 24.9 ± 1.3 ml; $P=0.627$). Seven
201 of the eleven subjects failed to distinguish between the rinse solutions. The remaining four
202 correctly differentiated the placebo from the two carbohydrate solutions, but only one
203 correctly distinguished between the 7% and 14% concentrations.

204

205 Discussion

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

211 The findings of this study support those of Wright and Davison (2013), who showed that
212 there was no additional performance benefit of mouth rinsing a 12% carbohydrate solution
213 over that observed between a 6% solution and a placebo. Wright and Davison (2013)
214 recruited 7 males who were instructed to cover as much distance as possible in a 90 min
215 treadmill test, rinsing their mouth at 0, 15, 30 and 45 min of the protocol. However, the
216 participants only covered relatively short distances (Placebo 13.9 ± 1.7 km; 6% CHO $14.6 \pm$
217 1.7 km; 12% CHO 14.9 ± 1.6 km), suggesting the population were not well trained, despite
218 being reported to be in competitive sports teams. Furthermore, extremely large performance
219 improvements seen in some trials (up to 18.6%) far exceed the typical improvements seen in
220 performance studies, calling into question either the standardisation of pre-trial conditions or
221 the variability of the protocol employed. The present study used the same cycling time trial
222 protocol as the original mouth rinse studies (Carter et al., 2004; Chambers et al., 2009), which
223 has a reported variability of 3.35 % in trained cyclists (Jeukendrup et al., 1996). As such, we
224 have confidence that the observed differences between performance trials in the present study
225 were a consequence of the carbohydrate rinse intervention.

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

229 as the present study and showed no effect of mouth rinsing with 4, 6, or 8% carbohydrate
230 (89% sucrose; 11% glucose) solutions compared to a 0% placebo. However, the cyclists had
231 ingested a meal 3 h prior to exercise and were therefore not in a fasted state during the trials
232 (Ispoglou et al., 2015). Although Lane et al. (2013) reported that mouth rinsing a 10%
233 maltodextrin solution for 10 s improved 60 min cycle time trial performance in both fed and
234 fasted conditions, the magnitude of improvement was greater in the fasted condition.
235 Furthermore, Beelen and colleagues (2009) have shown that 1 h cycling time trial performance
236 is not influenced by mouth rinsing a 6.4% maltodextrin solution compared to water when
237 cyclists ingest ~ 2.5 g carbohydrate \cdot kgBM⁻¹ two hours before the test. Indeed, imaging studies
238 have shown that the central activation of reward centres in the brain in response to
239 carbohydrate feedings are diminished under conditions of satiety in comparison to hunger
240 (Haase et al., 2009). Thus, **although providing a carbohydrate rich meal prior to exercise may**
241 **have some ecological validity**, it is not favourable to detecting small performance benefits
242 that carbohydrate mouth rinse may provide (Rollo et al., 2010).

243 More recently Kulaksiz et al. (2016) reported that 20 km cycle time trial performance was not
244 influenced by mouth rinsing either 3%, 6% or 12% **maltodextrin** solutions compared to a 0%
245 placebo. Direct comparisons to the present study are difficult due to differences in protocol
246 used and training status of the participants. Kulaksiz et al (2016) recognised that the $\dot{V}O_{2\max}$
247 values of their participants were lower (~ 21 - 42%) than those recruited to previous mouth
248 rinse studies (Carter et al., 2004; Chambers et al., 2009; Lane et al., 2013). Although
249 Kulaksiz et al. (2016) used a validated protocol (Zavorsky et al., 2007), it has been shown
250 that top performers (i.e., those cyclists that maintained a higher average power output over 20
251 km) had a coefficient of variation that was four times lower compared to the bottom
252 performers (1.2% and 4.8 %, respectively; Zavorsky et al., 2007). The mean power output in
253 the study by Kulaksiz et al. (2016) was lower (~ 200 Watts) than the bottom cyclists in the

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

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

277 The mechanism(s) by which endurance performance is improved by mouth rinsing and
278 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, orbitofrontal 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 flexion (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
314 authors approved the final version of the paper. No funding was received for this work. IR is
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.

318

319 **References**

- 320 Anantaraman, R., A. A. Carmines, G. A. Gaesser & A. Weltman (1995). Effects of carbohydrate
321 supplementation on performance during 1 hour of high-intensity exercise. *Int J Sports Med*
322 16(7): 461-465.
- 323 Ataide-Silva, T., T. Ghiarone, R. Bertuzzi, C. G. Stathis, C. G. Leandro & A. E. Lima-Silva (2016).
324 Carbohydrate Mouth Rinse Ameliorates Neuromuscular Response with Lower Endogenous
325 Carbohydrate Stores. *Med Sci Sports Exerc.* [Epub ahead of print].
- 326 Bastos-Silva, V. J., A. Melo Ade, A. E. Lima-Silva, F. A. Moura, R. Bertuzzi & G. G. de Araujo
327 (2016). Carbohydrate Mouth Rinse Maintains Muscle Electromyographic Activity and
328 Increases Time to Exhaustion during Moderate but not High-Intensity Cycling Exercise.
329 *Nutrients* 8(3).
- 330 Beelen, M., J. Berghuis, B. Bonaparte, S. Ballak, A. E. Jeukendrup & L. J. C. van Loon (2009).
331 Carbohydrate mouth rinsing in the fed state does not enhance time trial performance. *Int J*
332 *Sport Nutr Exerc Metab* 19(4): 400-409.
- 333 Below, P. R., R. Mora-Rodriguez, J. Gonzalez-Alonso & E. F. Coyle (1995). Fluid and carbohydrate
334 ingestion independently improve performance during 1 h of intense exercise. *Med Sci Sports*
335 *Exerc* 27(2): 200-210.
- 336 Berthoud, H. R. (2003). Neural systems controlling food intake and energy balance in the modern
337 world. *Curr Opin Clin Nutr Metab Care* 6(6): 615-620.
- 338 Borg, G. A. V. (1982). Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 14, 377-
339 381.
- 340 Carter, J., A. E. Jeukendrup, T. Mundel & D. A. Jones (2003). Carbohydrate supplementation
341 improves moderate and high-intensity exercise in the heat. *Pflugers Arch* 446(2): 211-219.
- 342 Carter, J. M., A. E. Jeukendrup & D. A. Jones (2004). The effect of carbohydrate mouth rinse on 1-h
343 cycle time trial performance. *Med Sci Sports Exerc* 36(12): 2107-2111.
- 344 Chambers, E. S., M. W. Bridge & D. A. Jones (2009). Carbohydrate sensing in the human mouth:
345 effects on exercise performance and brain activity. *J Physiol* 578.8: 1779-1794.
- 346 Coggan, A. R. & E. F. Coyle (1987). Reversal of fatigue during prolonged exercise by carbohydrate
347 infusion or ingestion. *J Appl Physiol* 63(6): 2388-2395.
- 348 Coyle, E. F., A. R. Coggan, M. K. Hemmert & J. L. Ivy (1986). Muscle glycogen utilization during
349 prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol* 61(1): 165-172.
- 350 Desbrow, B., S. Anderson, J. Barrett, E. Rao & M. Hargreaves (2004). Carbohydrate-electrolyte
351 feedings and 1 h time trial cycling performance. *Int J Sport Nutr Exerc Metab* 14(5): 541-549.
- 352 Gam, S., K. J. Guelfi & P. A. Fournier (2013). Opposition of carbohydrate in a mouth-rinse solution
353 to the detrimental effect of mouth rinsing during cycling time trials. *Int J Sport Nutr Exerc*
354 *Metab* 23(1): 48-56.
- 355 Gant, N., C. M. Stinear & W. D. Byblow (2010). Carbohydrate in the mouth immediately facilitates
356 motor output. *Brain Res* 1350: 151-158.
- 357 Haase, L., B. Cerf-Ducastel & C. Murphy (2009). Cortical activation in response to pure taste stimuli
358 during the physiological states of hunger and satiety. *Neuroimage* 44(3): 1008-1021.
- 359 Ispoglou, T., D. O'Kelly, A. Angelopoulou, M. Bargh, J. P. O'Hara & L. C. Duckworth (2015). Mouth
360 Rinsing With Carbohydrate Solutions at the Postprandial State Fail to Improve Performance
361 During Simulated Cycling Time Trials. *J Strength Cond Res* 29(8): 2316-2325.
- 362 Jeukendrup, A., F. Brouns, A. J. Wagenmakers & W. H. Saris (1997). Carbohydrate-electrolyte
363 feedings improve 1 h time trial cycling performance. *Int J Sports Med* 18(2): 125-129.
- 364 Jeukendrup, A., W. H. Saris, F. Brouns & A. D. Kester (1996). A new validated endurance
365 performance test. *Med Sci Sports Exerc* 28(2): 266-270.
- 366 Jeukendrup, A. E. (2004). Carbohydrate intake during exercise and performance. *Nutrition* 20(7-8):
367 669-677.
- 368 Jeukendrup, A. E., J. M. Carter & I. Rollo (2013). Carbohydrate mouth rinse: performance effects and
369 mechanisms. *Sports Science Exchange* #118 26(118): 1-8.
- 370 Kringelbach, M. L., I. E. de Araujo & E. T. Rolls (2004). Taste-related activity in the human
371 dorsolateral prefrontal cortex. *Neuroimage* 21(2): 781-788.

- 372 Kulaksiz, T. N., S. N. Kosar, S. Bulut, Y. Guzel, M. E. Willems, T. Hazir & H. H. Turnagol (2016).
373 Mouth Rinsing with Maltodextrin Solutions Fails to Improve Time Trial Endurance Cycling
374 Performance in Recreational Athletes. *Nutrients* 8(5).
- 375 Lane, S. C., S. R. Bird, L. M. Burke & J. A. Hawley (2013). Effect of a carbohydrate mouth rinse on
376 simulated cycling time-trial performance commenced in a fed or fasted state. *Appl Physiol*
377 *Nutr Metab* 38(2): 134-139.
- 378 Neuffer, P. D., D. L. Costill, M. G. Flynn, J. P. Kirwan, J. B. Mitchell & J. Houmard (1987).
379 Improvements in exercise performance: effects of carbohydrate feedings and diet. *J Appl*
380 *Physiol* 62(3): 983-988.
- 381 Pottier, A., J. Bouckaert, W. Gilis, T. Roels & W. Derave (2008). Mouth rinse but not ingestion of a
382 carbohydrate solution improves 1-h cycle time trial performance. *Scand J Med Sci Spor*
383 20(1): 105-111.
- 384 Rollo, I., M. Cole, R. Miller & C. Williams (2010). Influence of mouth rinsing a carbohydrate
385 solution on 1-h running performance. *Med Sci Sports Exerc* 42(4): 798-804.
- 386 Rollo, I. & C. Williams (2009). Influence of Ingesting a Carbohydrate-Electrolyte Solution Before
387 and During a 1-hr Running Performance Test. *Int J Sport Nutr Exerc Metab* 19(6): 645-658.
- 388 Rollo, I. & C. Williams (2011). Effect of mouth-rinsing carbohydrate solutions on endurance
389 performance. *Sports Med* 41(6): 449-461.
- 390 Rolls, E. T. (2007). Sensory processing in the brain related to the control of food intake. *Proc Nutr*
391 *Soc* 66(1): 96-112.
- 392 Sinclair, J., L. Bottoms, C. Flynn, E. Bradley, G. Alexander, S. McCullagh, T. Finn & H. T. Hurst
393 (2014). The effect of different durations of carbohydrate mouth rinse on cycling performance.
394 *Eur J Sport Sci* 14(3): 259-264.
- 395 Smeets, P. A., C. de Graaf, A. Stafleu, M. J. van Osch & J. van der Grond (2005). Functional MRI of
396 human hypothalamic responses following glucose ingestion. *Neuroimage* 24(2): 363-368.
- 397 Tsintzas, K. & C. Williams (1998). Human muscle glycogen metabolism during exercise. Effect of
398 carbohydrate supplementation. *Sports Med* 25(1): 7-23.
- 399 Tsintzas, O. K., C. Williams, L. Boobis & P. Greenhaff (1996). Carbohydrate ingestion and single
400 muscle fiber glycogen metabolism during prolonged running in men. *J Appl Physiol* 81: 801-
401 809.
- 402 Turner, C. E., W. D. Byblow, C. M. Stinear & N. Gant (2014). Carbohydrate in the mouth enhances
403 activation of brain circuitry involved in motor performance and sensory perception. *Appetite*
404 80: 212-219.
- 405 van Rijn, I., S. Griffioen-Roose, C. de Graaf & P. A. Smeets (2015). Neural Processing of Calories in
406 Brain Reward Areas Can be Modulated by Reward Sensitivity. *Front Behav Neurosci* 9: 371.
- 407 Widrick, J. J., D. L. Costill, W. J. Fink, M. S. Hickey, G. K. McConnell & H. Tanaka (1993).
408 Carbohydrate feedings and exercise performance: effect of initial muscle glycogen
409 concentration. *J Appl Physiol* 74: 2998-3005.
- 410 Wright BF & Davison G. (2013). Carbohydrate mouth rinse improves 1.5 h run performance: is there
411 a dose-effect? *Int J Exerc Sci* 6: 328-340.
- 412 Zavorsky, G. S., J. M. Murias, J. Gow, D. J. Kim, C. Poulin-Harnois, S. Kubow & L. C. Lands
413 (2007). Laboratory 20-km cycle time trial reproducibility. *Int J Sports Med* 28: 743-748.

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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 |

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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 \pm SD values. Bottom panel displays individual subject data. # denotes a
425 significant difference from PLA trial.

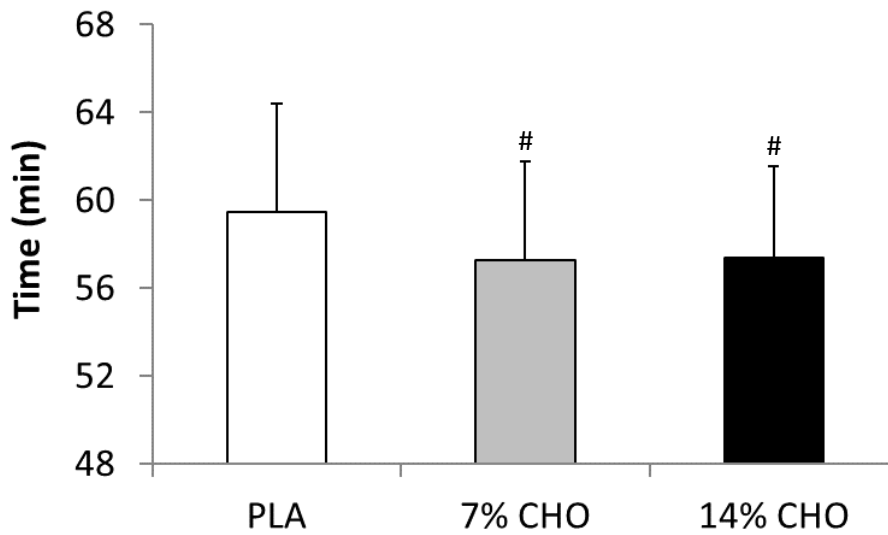
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427 Figure 2. Time to complete each 12.5% segment of the time trial in the PLA, 7% CHO and
428 14% CHO trials. Data are expressed as mean \pm SD. There was a main effect of time
429 ($P < 0.001$) and trial ($P < 0.001$), but no interaction effect.

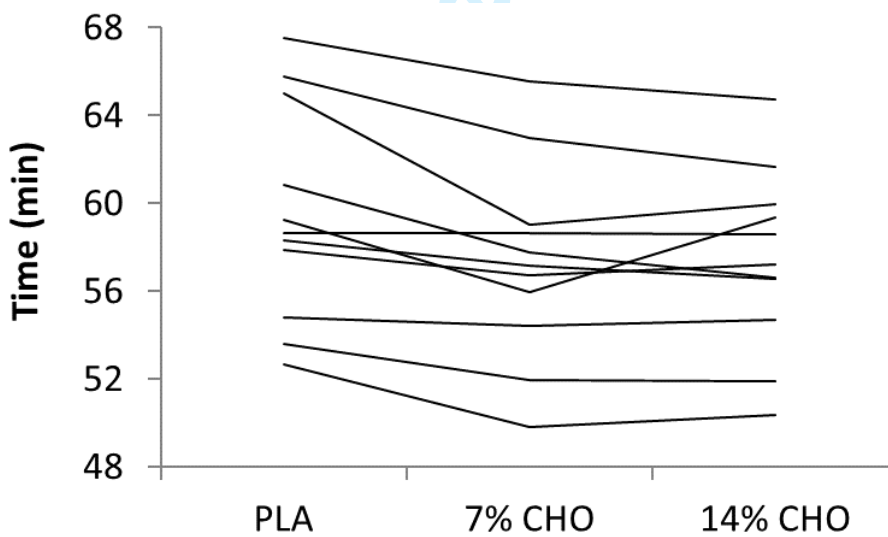
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431 **Figures**

432 Figure 1.

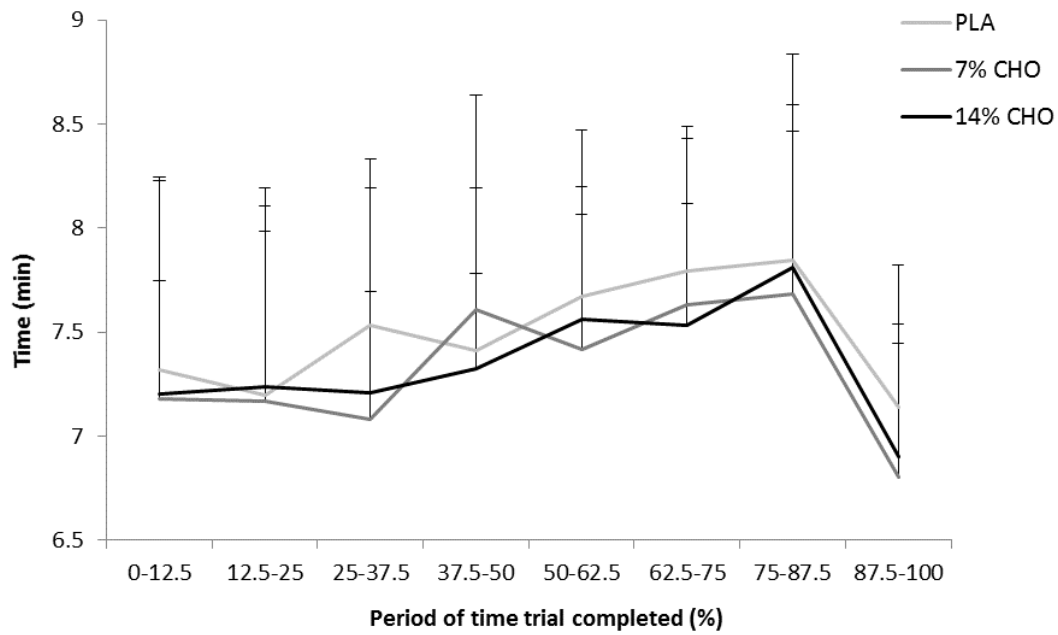


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435 Figure 2.



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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.