

Kizzi, Joseph, Sum, Alvin, Houston, Fraser E. and Hayes, Lawrence ORCID: <https://orcid.org/0000-0002-6654-0072> (2016) Influence of a caffeine mouth rinse on sprint cycling following glycogen depletion. *European Journal of Sport Science*, 16 (8). pp. 1087-1094.

Downloaded from: <http://insight.cumbria.ac.uk/id/eprint/2068/>

Usage of any items from the University of Cumbria's institutional repository 'Insight' must conform to the following fair usage guidelines.

Any item and its associated metadata held in the University of Cumbria's institutional repository Insight (unless stated otherwise on the metadata record) may be copied, displayed or performed, and stored in line with the JISC fair dealing guidelines (available [here](#)) for educational and not-for-profit activities

provided that

- the authors, title and full bibliographic details of the item are cited clearly when any part of the work is referred to verbally or in the written form
 - a hyperlink/URL to the original Insight record of that item is included in any citations of the work
- the content is not changed in any way
- all files required for usage of the item are kept together with the main item file.

You may not

- sell any part of an item
- refer to any part of an item without citation
- amend any item or contextualise it in a way that will impugn the creator's reputation
- remove or alter the copyright statement on an item.

The full policy can be found [here](#).

Alternatively contact the University of Cumbria Repository Editor by emailing insight@cumbria.ac.uk.

1 ORIGINAL INVESTIGATION

2

3 **Influence of a caffeine mouth rinse on sprint cycling following glycogen depletion**

4

5 Running head: Caffeine rinsing following glycogen depletion

6

7

8

9

10 **Abstract**

11 Attenuated performance during intense exercise with limited endogenous
12 carbohydrate (CHO) is well documented. Therefore, this study examined whether caffeine
13 (CAF) mouth rinsing would augment performance during repeated sprint cycling in
14 participants with reduced endogenous CHO. Eight recreationally active males (aged 23 ± 2
15 yr, body mass 84 ± 4 kg, stature 178 ± 7 cm) participated in this randomized, single-blind,
16 repeated-measures crossover investigation. Following familiarization, participants attended
17 two separate evening glycogen depletion sessions. The following morning, participants
18 completed five, 6 s sprints on a cycle ergometer (separated by 24 s active recovery), with
19 mouth rinsing either 1) a placebo solution or 2) a 2% caffeine solution. During a fifth visit,
20 participants completed the sprints without prior glycogen depletion. Repeated measures
21 ANOVA identified significant main effect of condition (CAF, placebo, and control [$P < 0.05$;
22 $ES = 0.850-0.897$]), sprint (1-5 [$P < 0.005$; $ES = 0.871-0.986$]), and interaction (condition x
23 sprint [$P < 0.05$; $ES = 0.831-0.846$]), for peak and mean power. The control condition exhibited
24 the highest peak power (overall mean 760 ± 77 W) and mean power (overall mean $699 \pm$
25 83 W) over the five sprints ($P < 0.001$ in both instances). CAF peak power (overall mean $643 \pm$
26 79 W) was significantly greater than placebo (mean 573 ± 79 W [$P < 0.05$; $ES = 0.850$]).
27 Additionally, CAF mean power (overall mean 589 ± 80 W) was significantly greater than
28 placebo (519 ± 82 W [$P < 0.05$; $ES = 0.397$]). These data indicate that mouth rinsing a
29 caffeinated solution reduces decrements caused by CHO reduction, which may benefit
30 athletes wishing to train in a low-CHO state.

31

32 **Key words:** Anaerobic · Carbohydrate · Ergogenic · High intensity · Repeated sprint
33 exercise

34

35 **Introduction**

36 That exercise performance is attenuated with low carbohydrate (CHO) availability,
37 yet certain training adaptations are enhanced with low endogenous CHO, presents a challenge
38 to athletes aiming to maximize training quality (Impey et al., 2015). Exogenous supply of
39 CHO has the potential to improve exercise performance (Stellingwerff & Cox, 2014; Wilson,
40 2015), particularly high-intensity exercise that is more reliant on CHO than fat oxidation
41 (Spriet, 2014). Moreover, numerous studies have described attenuated performance during
42 high-intensity exercise when endogenous CHO availability is limited (Gavin, Myers, &
43 Willems 2015a; 2015b). For example, Silva-Cavalcante and colleagues (2013) reported that
44 when endogenous CHO availability was reduced by ~30%, 4 km cycling time trial (TT) time
45 was 2.1% slower than in a control condition. Furthermore, Langfort, Zarzeczny, Pilis, Nazar,
46 and Kaciuba-Uscitko (1997) observed reduced mean power during a 30 s Wingate test (from
47 581 ± 7 to 533 ± 7 W) in healthy men after three days of a low CHO diet (~5% CHO)
48 compared with a normal diet (~50% CHO). Paradoxically, it is commonplace for some
49 athletes to train in a state of low CHO availability (Taylor et al., 2013; Impey et al., 2015) to
50 augment molecular signalling for endurance training adaptations (Bartlett, Hawley, &
51 Morton, 2015). For example, in an elegant investigation, Hansen et al. (2004) examined the
52 influence of performing one leg knee-extensor exercise in a state of high or low muscle
53 glycogen for 10 weeks. This was achieved by training one leg twice a day, every second day,
54 and training the contralateral leg once daily. These authors reported that following training,
55 time to exhaustion was markedly improved in the low-glycogen leg, compared to the high-
56 glycogen leg. Furthermore, activity of the mitochondrial enzyme 3-hydroxyacyl-CoA
57 dehydrogenase and resting muscle glycogen was augmented following training, but to a
58 greater extent in the low-glycogen leg, which suggests enhanced skeletal muscle oxidative
59 capacity following training with limited endogenous CHO. Additionally, Cochran et al.

60 (2015) investigated the influence of low CHO intake between high intensity interval training
61 sessions performed three hours apart. Improved time trial time performance was observed
62 after only two weeks in the group consuming $0.3 \text{ g}\cdot\text{kg}^{-1}$ CHO between sessions ($211 \pm 66 \text{ W}$
63 to $244 \pm 75 \text{ W}$), compared to the group consuming $2.3 \text{ g}\cdot\text{kg}^{-1}$ CHO between sessions ($203 \pm$
64 53 W to $219 \pm 60 \text{ W}$). Taken together, these investigations support the notion that exercise
65 training performed in a CHO-restricted state may enhance skeletal muscle adaptations which
66 in turn increase work capacity.

67 It has long been known that the oralpharyngeal cavity contains receptors that respond
68 to taste (Beidler, 1954). However, until recently it was thought improved exercise
69 performance following ingestion of substrates was solely due to post-absorptive effects
70 (Burke & Maughan, 2015). It is now recognized the response to substrate ingestion begins in
71 the mouth, via specific receptors, and continues in the gut, via the release of various
72 hormones influencing substrate metabolism (Burke & Maughan, 2015; Hagger &
73 Chatzisarantis, 2013). Indeed, Kamimori et al. (2002) observed a significantly greater
74 caffeine absorption rate following administration of caffeinated chewing gum, compared to
75 capsule formulation. These authors therefore concluded the buccal mucosa was a primary site
76 for caffeine absorption into systemic circulation, as a result of caffeine-adenosine receptor
77 interactions within the mouth (Rubinstein, Chandilawa, Dagar, Hong, & Gao, 2001).
78 Subsequent investigations have found improved performance in aerobic (Doering, Fell,
79 Leveritt, Desbrow, & Shing, 2014; Pataky et al., 2015), anaerobic (Kasper et al., 2015), and
80 repeated sprint (Beaven, Maulder, Pooley, Kilduff, & Cook, 2013; Correia-Oliveira et al.,
81 2014) exercise following caffeine mouth rinsing. However, these results may depend on
82 testing methods, as Clarke, Kornilios, and Richardson (2015) recently reported that caffeine
83 (CAF) mouth rinsing did not improve muscular strength or muscular endurance during the
84 bench press exercise.

85 Caffeine ingestion has previously demonstrated efficacy in reducing impairments in
86 running (Kasper et al., 2015) and cycling (Silva-Cavalcante et al., 2013) performance, caused
87 by a CHO-lowering protocol. Kasper and colleagues (2015) investigated high-intensity
88 interval running capacity (1 min intervals at 80% maximal oxygen uptake, interspersed with 1
89 min walking at 6 km·h⁻¹). These authors reported improved running capacity (measured by
90 total distance covered until fatigue) when CAF ingestion was added to a CHO mouth rinse in
91 a glycogen depleted state. The practical application of this information is that athletes can
92 recover performance decrements caused by low endogenous CHO with administration of
93 CAF. However, there are a paucity of data concerning the effect of mouth rinsing a solution
94 containing solely CAF on repeated sprint performance with low endogenous CHO
95 availability. Therefore, the objective of this investigation was to examine whether CAF
96 mouth rinsing would rescue performance reductions caused by low endogenous CHO
97 availability during repeated sprint cycling, compared to placebo.

98

99 **Materials and methods**

100 *Subjects*

101 Eight recreationally active males (aged 23 ± 2 yr, body mass 84 ± 4 kg, stature 178 ±
102 7 cm, maximal power output [W_{max}]194 ± 17 W) participated in this randomized, single-blind
103 and repeated-measures crossover investigation. Participants gave written informed consent
104 and the investigation was approved by the London Metropolitan University Ethical Review
105 Committee. Participants were free from medication, and abstained from exercise, caffeinated
106 beverages, and alcohol for the previous 24 h.

107

108 *Design*

109 Participants visited the laboratory on six occasions. On the first visit, athletes
110 underwent anthropometric assessment and an incremental test followed by a repeated sprint
111 cycling familiarization trial. Participants then attended two separate glycogen depletion
112 sessions (commencing between 17.30 – 20.00 h) followed by five, 6 s sprint cycling bouts
113 (each separated by 24 s active recoveries) the following morning (08.00 – 09.00 h). During a
114 further visit, participants completed the repeated sprint cycling bouts without prior glycogen
115 depletion (six visits in total; Figure 1).

116

117 *****INSERT FIGURE 1 NEAR HERE*****

118

119 *Incremental Test*

120 The incremental test was performed on a cycle ergometer (Wattbike trainer, Wattbike
121 Ltd., Nottingham, UK) and consisted of a 3 min warm-up at 100 W, followed by increments
122 of 30 W every 3 min, until voluntary exhaustion, or when participants were unable to
123 maintain the required power output (Bentley et al. 2007). Maximal power output (W_{\max}) was
124 defined as the highest power output maintained during a complete 3 min stage. When the last
125 stage was not completed, W_{\max} was determined in accordance with the methods of Kuipers,
126 Verstappen, Keizer, Geurten, & van Kranenburg (1985).

127

128 *Carbohydrate Availability Lowering Protocol*

129 Participants arrived at the laboratory between 17.30 – 20.00 h, at least two hours
130 postprandial. The protocol used for reducing endogenous CHO availability has previously
131 been validated and shown to reduce endogenous CHO availability to 30% of pre-exercise
132 values (Gollnick, Piehl, & Saltin, 1974). The protocol consisted of a constant power output,

133 at an intensity corresponding to 70% W_{\max} for 90 min on a cycle ergometer (Wattbike trainer,
134 Wattbike Ltd., Nottingham, UK). After 5 min rest, participants performed six, 1 min cycling
135 bouts at 125% W_{\max} , with 1 min rest intervals.

136

137 *Dietary Control*

138 During the morning and afternoon of the CHO availability lowering protocol,
139 participants followed the same dietary pattern contained in their food record, up to the
140 beginning of exercise. This was determined using a food diary on the day prior to, and the
141 day of, the incremental test and familiarization with the sprint cycling protocol. After the
142 exercise protocol was finished (19.15 – 21.45 h), participants received a low-CHO meal
143 replacement (400 ml; total energy 97 kcal, 0.6 g CHO, 0.3 g fat, and 23.0 g protein
144 [MyProtein, The Hut.com Ltd, UK]). Participants received the same standardized, low-CHO
145 meal replacement one hour before the trial the next morning (~08.00 h). In the control (CON)
146 trial, participants were asked to replicate the diet recorded 24 hours before the familiarization
147 visit, and consumed a standardized meal derived from their diet record. According to self-
148 reporting, all participants adhered to dietary replication.

149

150 *Repeated Sprint Cycling Test*

151 During morning visits, participants performed five, 6 s cycling sprints under the
152 following conditions: 1) 12–14 h after a validated exercise-protocol designed to reduce
153 endogenous CHO availability, followed by placebo (PLA) mouth rinsing, 2) 12–14 h after a
154 validated exercise-protocol designed to reduce endogenous CHO availability, followed by
155 CAF mouth rinsing, and 3) with no prior depletion or mouth rinse (CON). Randomization
156 was ensured by assigning each condition a number (1-3), then generating eight sets (one per
157 participant) of randomized 1, 2, and 3, using a computer program (Research randomizer:

158 Version 4.0). For example, if participant one received '1, 2, 3' they would conduct the
159 conditions in the following order: CON, PLA, CHO and if participant two received '2, 1, 3'
160 they would conduct the conditions in the following order: PLA, CON, CHO. Each visit was
161 separated by seven days for washout. Participants completed a standardized 5 min warm up at
162 100 W on a cycle ergometer (Monark 994E, Monark, Sweden), subsequently mouth rinsing
163 the solution for 10 s, before expectorating into a waste container. Participants mouth rinsed
164 between each 6 s sprint (six mouth rinses in total). Solutions consisted of 25 ml of a 2%
165 caffeine solution (CAF [500 mg; 6 mg·kg⁻¹]) or a taste-matched non-caloric placebo (PLA) in
166 line with previous investigations (Beaven et al., 2013). Placebo and CAF were taste matched
167 by using very strong **sugar-free** orange squash. Successful blinding of solutions was
168 confirmed by participants correctly guessing the administered solution on 10 of the 16
169 opportunities (Fisher's exact test P=0.376). Participants were required to pedal at 50 rpm
170 before being given a verbal countdown to start five, 6 s maximal sprint efforts with resistance
171 of 10% body mass applied to the flywheel, interspersed by 24 s active recovery (unloaded
172 pedaling) whereby participants repeated the 10 s mouth rinsing (as used by Beaven et al.,
173 2013). Mean power output and peak power output were recorded using the inbuilt software
174 (Monark 994E, Monark, Sweden) and verbal encouragement was given throughout.

175 Participants were asked to provide pain perception ratings following each sprint
176 (Cook, O'Connor, Eubanks, Smith, & Lee, 2007). A ten-point scale accompanied with
177 verbal, written and visual descriptions was used. This was chosen as high intra-class
178 correlations (r=.88-.98) suggest this scale is a reliable measure of pain perception during
179 exercise (Cook et al., 2007). Standardized verbal instruction of the correct use of the scale
180 was provided prior to each experimental procedure.

181

182 *Data Analysis*

183 Data were analyzed using SPSS Statistics version 20 (IBM North America, New
184 York, USA). To determine parametricity, Levene's tests (homogeneity of variance) and
185 Shapiro-Wilk (normal distribution) were employed. Where parametric assumptions were met,
186 data were analyzed using a 3 x 5 (condition x sprint) repeated measures analysis of variance
187 (ANOVA) to test for differences in peak and mean power, and perceived pain. Where an
188 interaction effect was detected, one-way ANOVA with Bonferoni correction was used to
189 detect between which condition differences existed. Significance was set *a priori* at $P < 0.05$
190 and effect sizes (ES) are reported for primary outcome measures in line with previous
191 recommendations (Cohen, 1992; Lakens, 2013).

192

193 **Results**

194 There was a significant main effect of condition, bout, and an interaction effect for
195 peak power output, mean power output, and perceived pain (all $P < 0.001$; $ES = 0.831-0.986$).
196 The CON condition exhibited the greatest peak power output (overall mean 760 ± 77 W; 95%
197 $CI = 712-808$ W) and mean power output (overall mean 699 ± 83 W; 95% $CI = 640-758$ W)
198 over the five sprints. There was an improvement in peak power (overall mean 573 ± 79 W;
199 95% $CI = 516-631$ W and 643 ± 79 W; 95% $CI = 582-705$ W for PLA and CAF respectively)
200 and mean power (overall mean 519 ± 82 W; 95% $CI = 450-578$ W and 589 ± 80 W; 95%
201 $CI = 521-657$ W for PLA and CAF respectively) following depletion and CAF compared to
202 depletion and PLA (Figure 2A;B). The CON condition exhibited the lowest perceived pain
203 (overall mean 4 ± 1) over the five sprints. There was a significant increase in perceived pain
204 following depletion and PLA compared to depletion and CAF (8 ± 1 and 7 ± 1 respectively
205 [Figure 2C]). Under CON and PLA conditions, peak power decreased by ~16% and ~17%
206 over the six bouts. Moreover, under CON and PLA conditions mean power decreased by

207 ~16% and ~20% over the six bouts. Under the CAF condition, participants maintained mean
208 power and peak power from bout one to five.

209

210 *Peak Power Output*

211 During sprint one, CON peak power (828 ± 51 W) was significantly greater than CAF
212 (615 ± 79 W; $P < 0.001$; $ES = 0.850$) and PLA (627 ± 68 W; $P < 0.001$; $ES = 0.859$). During
213 sprint two, CON peak power output (803 ± 63 W) was significantly greater than CAF ($617 \pm$
214 93 W; $P = 0.018$; $ES = 0.763$) and PLA (609 ± 65 W; $P = 0.004$; $ES = 0.836$). During sprint three,
215 CON peak power output (744 ± 73 W) was greater than CAF (631 ± 83 W; $P = 0.018$;
216 $ES = 0.583$) and PLA, (573 ± 71 W; $P = 0.004$; $ES = 0.766$), whilst CAF peak power output was
217 greater than PLA ($P = 0.015$; $ES = 0.352$). During sprint four, CON peak power output ($727 \pm$
218 62 W) and was greater than PLA, (542 ± 76 W; $P = 0.004$; $ES = 0.802$), but not CAF (654 ± 71
219 W; $P = 0.148$; $ES = 0.474$), whilst CAF peak power output was greater than PLA ($P = 0.001$;
220 $ES = 0.612$). During sprint five, CON peak power output (697 ± 63 W) was significantly
221 greater than PLA, (518 ± 74 W; $P = 0.005$; $ES = 0.805$), whilst CAF peak power output ($694 \pm$
222 54 W) was also greater than PLA ($P < 0.001$; $ES = 0.825$).

223

224 *Mean Power Output*

225 During sprint one, CON mean power output (757 ± 72 W) was significantly greater
226 than CAF (575 ± 82 W) and PLA ($[578 \pm 68$ W] $P = 0.001$; $ES = 0.765-0.789$). During sprint
227 two, CON mean power output (740 ± 84 W) was significantly greater than CAF (576 ± 90 W;
228 $P = 0.004$; $ES = 0.709$) and PLA (561 ± 71 W; $P = 0.002$; $ES = 0.780$). During sprint three, CON
229 mean power output (694 ± 75 W) was greater than CAF (583 ± 87 W; $P = 0.014$; $ES = 0.561$)

230 and PLA, (510 ± 85 W; $P=0.002$; $ES=0.754$), whilst CAF mean power output was greater
231 than PLA ($P=0.002$; $ES=0.393$). During sprint four, CON mean power output (665 ± 62 W)
232 and was greater than PLA, (483 ± 76 W; $P=0.002$; $ES=0.794$), whilst CAF mean power
233 output (596 ± 79 W) was also greater than PLA ($P<0.001$; $ES=0.587$). During sprint five,
234 CON mean power output (639 ± 75 W) was significantly greater than PLA, (461 ± 58 W;
235 $P=0.003$; $ES=0.798$), whilst CAF mean power output (617 ± 75 W) was significantly greater
236 than PLA ($P<0.001$; $ES=0.759$).

237

238 *****INSERT FIGURE 2 NEAR HERE*****

239

240 *Rating of perceived pain*

241 During sprint one, CON perceived pain (2 ± 1) was significantly less than CAF ($5 \pm$
242 1 ; $P=0.001$; $ES=0.853$) and PLA (6 ± 1 $P=0.001$; $ES=0.895$). During sprint two, CON
243 perceived pain (3 ± 1) was less than CAF (6 ± 1 ; $P=0.001$; $ES=0.853$) and PLA (7 ± 2 ;
244 $P=0.001$; $ES=0.896$). During sprint three, CON perceived pain (4 ± 1) was less than CAF (7
245 ± 1 ; $P=0.008$; $ES=0.808$) and PLA, (8 ± 1 ; $P=0.001$; $ES=0.932$). During sprint four, CON
246 perceived pain (4 ± 1) was less than CAF (7 ± 2 W; $P=0.003$; $ES=0.808$), and PLA, (9 ± 2 ;
247 $P<0.001$; $ES=0.932$), whilst CAF was less than PLA ($P=0.043$; $ES=0.578$). During sprint
248 five, CON perceived pain (5 ± 1) was significantly less than CAF (8 ± 2 W; $P=0.002$;
249 $ES=0.855$), and PLA, (9 ± 1 ; $P<0.001$; $ES=0.999$), whilst CAF perceived pain (8 ± 1) was
250 significantly less than PLA ($P=0.008$; $ES=0.688$).

251

252 **Discussion**

253 This study investigated the influence of reduced endogenous CHO on repeated sprint
254 cycling performance, and the effect CAF mouth rinsing had on performance in this state. The
255 primary finding was that mouth rinsing a caffeinated solution maintained repeated sprint
256 cycling performance in participants with reduced endogenous CHO availability compared to
257 control, whereas performance progressively decreased when mouth rinsing PLA. It is
258 important to note the temporal power profiles however, as CAF peak and mean power output
259 was not significantly greater compared to PLA until sprint three. Moreover, although CAF
260 mean power and peak power was not significantly different from CON during sprints three to
261 six, reduced performance compared to CON was observed during sprints one and two.

262 Results reported here are in line with previous investigations suggesting that a) CAF
263 mouth rinsing can improve repeated sprint exercise performance (Beaven et al., 2013), and b)
264 CAF can reduce deleterious performance effects of glycogen depletion (Silva-Cavalcante et
265 al., 2013; Kasper et al., 2015). Beaven et al. (2013) recently reported that when compared to
266 placebo, CAF mouth rinsing improved peak and mean cycling power during sprint one and
267 two (of five), yet reduced mean power during the final sprint. These authors suggested a role
268 for caffeine in activating a supraspinal or central mechanism, capable of enhancing neural
269 drive to motor units, accessing muscle recruitment reserve. As such, this additional muscle
270 recruitment may have led to rapid depletion of ATP, evidenced by a reduction in mean power
271 during the final sprint. Although our data agree, in part, with Beaven and colleagues (2013) in
272 reporting increased peak and mean power following CAF mouth rinsing, no fatiguing effect
273 was observed as a result of increased power profiles. Therefore, we attribute this
274 phenomenon to the influence of glycogen depletion in the present investigation. i.e. low
275 endogenous CHO availability did not permit recruitment of the muscle recruitment reserve.
276 In support, Kasper et al. (2015) previously observed that the addition of a 200 mg CAF dose
277 improved high intensity interval running capacity in a CHO restricted state compared to

278 solely a CHO mouth rinse (65 ± 26 min compared to 52 ± 23 min). Moreover, both these
279 conditions were superior to placebo (36 ± 22 min) indicating that CHO mouth rinsing
280 abrogates the deleterious effect of low endogenous CHO, and that the addition of CAF
281 ingestion has an additive effect.

282 Whilst we accept the present investigation as descriptive, rather than mechanistic, one
283 potential mechanism by which CAF improved power profiles is a reduction in pain
284 perception (Duncan, Stanley, Parkhouse, Cook, & Smith, 2013; Meeusen, Roelands, &
285 Spriet, 2013). Gonglach, Ade, Bemben, Larson, and Black (2015) suggested caffeine
286 ingestion exerts an ergogenic effect by allowing greater work to be performed for a given
287 amount of perceived pain at moderate intensity. This is supported by data in the present
288 investigation whereby peak and mean power output was significantly increased under the
289 CAF condition compared to PLA, despite a reduction in perceived pain. Moreover, numerous
290 authors have described a dampening of pain perception (Duncan & Oxford, 2012), or
291 enhanced athletic performance for equal pain perception (Astorino, Terzi, Roberson, &
292 Burnett, 2011; Astorino, Roupoli, & Valdivieso, 2012) during exercise with CAF compared
293 to placebo. Taken together, these data suggest muscle pain exerts an effect in the regulation
294 of exercise intensity (Delextrat et al., 2015), and caffeine supplementation (whether by
295 ingestion [Gonglach et al., 2015], or mouth rinsing [as in the present study]) modifies
296 perception of pain. A second potential mechanism for improved performance within the
297 present study was that CAF increased voluntary muscle activation. Behrens and colleagues
298 (2015b) observed $7 \text{ mg}\cdot\text{kg}^{-1}$ CAF increased rate of torque development and enhanced
299 normalized muscle activity in the agonist muscles (plantar flexors) during maximal isometric
300 voluntary contraction, without accompanying alteration to antagonist muscle activity. The
301 same research group (Behrens et al., 2015a) reported a similar phenomenon in the knee
302 extensors, as $8 \text{ mg}\cdot\text{kg}^{-1}$ CAF increased maximal voluntary torque and muscle activation

303 during concentric, isometric, and eccentric contractions. As such, increased muscle activation
304 may explicate improved power profiles within the present study, however this is a *posteoiri*
305 hypothesis, and should be interpreted with caution, as electromyography was outside the
306 scope of the present investigation.

307 The practical application of the present study is that performance during repeated
308 sprint cycling with reduced endogenous CHO can be improved by mouth rinsing a
309 caffeinated solution, rather than ingestion of fluid or chewing gum, which may be preferential
310 to some athletes. Therefore, we believe our data to have practical implications for those
311 sportspersons who purposely include periods of CHO-restriction into their training
312 programmes to strategically enhance muscle oxidative capacity, in the form of mitochondrial
313 adaptations.

314 In conclusion, we provide novel data demonstrating that mouth rinsing a caffeinated
315 solution when in a CHO-depleted state ameliorates low CHO-induced sprint cycling
316 performance decrements. Future research may wish to explore the chronic adaptations to high
317 intensity sprint training with reduced CHO, with and without a caffeinated mouth rinse, and
318 compared to training in a state of high CHO availability.

319

320 Authors report no funding sources or conflict of interests.

321

322 **References**

323 Astorino, T. A., Roupoli, L. R., & Valdivieso, B. R. (2012). Caffeine does not alter RPE or
324 pain perception during intense exercise in active women. *Appetite*, *59*, 585-90. doi:
325 10.1016/j.appet.2012.07.008

326 Astorino, T. A., Terzi, M. N., Roberson, D. W., & Burnett, T. R. (2011). Effect of caffeine
327 intake on pain perception during high-intensity exercise. *International Journal of Sports*
328 *Nutrition and Exercise Metabolism*, 21, 27-32.

329 Bartlett, J. D., Hawley, J. A., & Morton, J. P. (2015). Carbohydrate availability and exercise
330 training adaptation: too much of a good thing? *European Journal of Sports Science*, 15, 3-12.
331 doi: 10.1080/17461391.2014.920926

332 Beaven, C. M., Maulder, P., Pooley, A., Kilduff, L., & Cook, C. (2013). Effects of caffeine
333 and carbohydrate mouth rinses on repeated sprint performance. *Applied Physiology,*
334 *Nutrition, and Metabolism*, 38, 633-7. doi: 10.1139/apnm-2012-0333

335 Behrens, M., Mau-Moeller, A., Weippert, M., Fuhrmann, J., Wegner, K., Skripitz, R., Bader,
336 R., & Bruhn, S. (2015a). Caffeine-induced increase in voluntary activation and strength of the
337 quadriceps muscle during isometric, concentric and eccentric contractions *Scientific Reports*,
338 5, 10209. doi: 10.1038/srep10209

339 Behrens, M., Mau-Moeller, A., Heise, S., Skripitz, R., Bader, R., & Bruhn, S. (2015b).
340 Alteration in neuromuscular function of the plantar flexors following caffeine ingestion
341 *Scandinavian Journal of Medicine and Science in Sports*, 25, e50-7. doi: 10.1111/sms.12243

342 Beidler, L. M. (1954). A theory of taste stimulation. *Journal of General Physiology*, 38, 133-
343 9.

344 Bentley, D. J., Newell, J., & Bishop, D. (2007). Incremental exercise test design and analysis:
345 implications for performance diagnostics in endurance athletes. *Sports Medicine*, 37, 575-86.
346 doi: 10.2165/00007256-200737070-00002

347 Burke, L. M. & Maughan, R. J. (2015). The Governor has a sweet tooth - mouth sensing of
348 nutrients to enhance sports performance. *European Journal of Sports Science*, 15, 29-40. doi:
349 10.1080/17461391.2014.971880

350 Clarke, N. D., Kornilios, E., & Richardson, D. L. (2015). Carbohydrate and caffeine mouth
351 rinses do not affect maximum strength and muscular endurance performance. *Journal of*
352 *Strength and Conditioning Research*, 29, 1926-31. doi: 10.1519/JSC.0000000000000945

353 Cochran, A. J., Myslik, F., MacInnis, M. J., Percival, M. E., Bishop, D., Tarnapolsky, M. A.,
354 & Gibala, M. J. (2015). Manipulating Carbohydrate Availability Between Twice-Daily
355 Sessions of High-Intensity Interval Training Over 2 Weeks Improves Time-Trial
356 Performance. *International Journal of Sports Nutrition and Exercise Metabolism*, 25, 463-70.
357 doi: 10.1123/ijsnem.2014-0263

358 Cohen, J. (1992). A power primer. *Psychological Bulletin*, 112, 155-9.

359 Cook, D., O'Connor, P., Eubanks, S., Smith, J., & Lee, M. (2007). Naturally occurring
360 muscle pain during exercise: assessment and experimental evidence. *Medicine and Science in*
361 *Sports and Exercise*, 29, 999-1012.

362 Correia-Oliveira, C. R., Santos, R. A., Silva-Cavalcante, M. D., Bertuzzi, R., Kiss, M. A.,
363 Bishop, D. J., & Lima-Silva, A. E. (2014). Prior low- or high-intensity exercise alters pacing
364 strategy, energy system contribution and performance during a 4-km cycling time trial. *PLoS*
365 *One*, 9, e110320. doi: 10.1371/journal.pone.0110320

366 Delextrat, A., O'Connor Ellis, M., Baker, C. E., Matthew, D., Sum, A., & Hayes, L. D.
367 (2015). Acetaminophen ingestion improves repeated sprint cycling performance in females:
368 A randomized crossover trial. *Kinesiology*, 47, 145-50.

369 Doering, T. M., Fell, J. W., Leveritt, M. D., Desbrow, B., & Shing, C. M. (2014). The effect
370 of a caffeinated mouth-rinse on endurance cycling time-trial performance. *International*
371 *Journal of Sports Nutrition and Exercise Metabolism*, 24, 90-7. doi: 10.1123/ijsnem.2013-
372 0103

373 Duncan, M. J. & Oxford, S. W. (2012). Acute caffeine ingestion enhances performance and
374 dampens muscle pain following resistance exercise to failure. *Journal of Sports Medicine and*
375 *Physical Fitness*, 52, 280-5.

376 Duncan, M. J., Stanley, M., Parkhouse, N., Cook, K., & Smith, M. (2013). Acute caffeine
377 ingestion enhances strength performance and reduces perceived exertion and muscle pain
378 perception during resistance exercise. *European Journal of Sports Science*, 13, 392-9. doi:
379 10.1080/17461391.2011.635811

380 Gavin, J. P., Myers, S. D., & Willems, M. E. (2015a). The effect of glycogen reduction on
381 cardiorespiratory and metabolic responses during downhill running. *European Journal of*
382 *Applied Physiology*, 115, 1125-33. doi: 10.1007/s00421-014-3094-4

383 Gavin, J. P., Myers, S. D., & Willems, M. E. (2015b). Neuromuscular responses to mild-
384 muscle damaging eccentric exercise in a low glycogen state. *Journal of Electromyography*
385 *and Kinesiology*, 25, 53-60. doi: 10.1016/j.jelekin.2014.10.005

386 Gollnick, P. D., Piehl, K., & Saltin, B. 1974. Selective glycogen depletion pattern in human
387 muscle fibres after exercise of varying intensity and at varying pedalling rates. *Journal of*
388 *Physiology*, 241, 45-57.

389 Gonglach, A. R., Ade, C. J., Bembien, M. G., Larson, R. D., & Black, C. D. (2015). Muscle
390 Pain as a Regulator of Cycling Intensity: Effect of Caffeine Ingestion. *Medicine and Science*
391 *in Sports and Exercise*, Epub ahead of print. doi: 10.1249/MSS.0000000000000767

392 Hagger, M. S. & Chatzisarantis, N. L. (2013). The sweet taste of success: the presence of
393 glucose in the oral cavity moderates the depletion of self-control resources. *Personality and*
394 *Social Psychology Bulletin*, 39, 28-42. doi: 10.1177/0146167212459912

395 Hansen, A. K., Fischer, C. P., Plomgaard, P., Andersen, JL., Saltin, B., & Pedersen, BK.
396 (2005). Skeletal muscle adaptation: training twice every second day vs. training once daily.
397 *Journal of Applied Physiology*, 98, 93-99. doi: 10.1152/jappphysiol.00163.2004

398 Impey, S. G., Smith, D., Robinson, A. L., Owens, D. J., Bartlett, J. D., Smith, K., ... Morton,
399 J. P. (2015). Leucine-enriched protein feeding does not impair exercise-induced free fatty
400 acid availability and lipid oxidation: beneficial implications for training in carbohydrate-
401 restricted states. *Amino Acids*, *47*, 407-16. doi: 10.1007/s00726-014-1876-y

402 Kamimori, G. H., Karyekar, C. S., Otterstetter, R., Cox, D. S., Balkin, T. J., Belenky, G. L.,
403 & Eddington, N. D. (2002). The rate of absorption and relative bioavailability of caffeine
404 administered in chewing gum versus capsules to normal healthy volunteers. *International*
405 *Journal of Pharmacology*, *234*, 159-67. doi: 10.1016/S0378-5173(01)00958-9

406 Kasper, A. M., Cocking, S., Cockayne, M., Barnard, M., Tench, J., Parker, L., ... Morton, J.
407 P. (2015). Carbohydrate mouth rinse and caffeine improves high-intensity interval running
408 capacity when carbohydrate restricted. *European Journal of Sports Science*, Epub ahead of
409 print. doi: 10.1080/17461391.2015.1041063

410 Kuipers, H., Verstappen, F. T., Keizer, H. A., Geurten, P., & van Kranenburg, G. (1985).
411 Variability of aerobic performance in the laboratory and its physiologic correlates.
412 *International Journal of Sports Medicine*, *6*, 197-201.

413 Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a
414 practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*, 863. doi:
415 10.3389/fpsyg.2013.00863

416 Langfort, J., Zarzeczny, R., Pilis, W., Nazar, K., & Kaciuba-Uscitko, H. (1997). The effect of
417 a low-carbohydrate diet on performance, hormonal and metabolic responses to a 30-s bout of
418 supramaximal exercise. *European Journal of Applied Physiology and Occupational*
419 *Physiology*, *76*, 128-33.

420 Meeusen, R., Roelands, B., & Spriet, L. L. (2013). Caffeine, exercise and the brain. *Nestle*
421 *Nutrition Institute Workshop Series*, *76*, 1-12. doi: 10.1159/000350223

422 Pataky, M. W., Womack, C. J., Saunders, M. J., Goffe, J. L., D'Lugos, A. C., El-Sohemy, A.,
423 & Luden, N. D. (2015). Caffeine and 3-km cycling performance: Effects of mouth rinsing,
424 genotype, and time of day. *Scandinavian Journal of Medicine and Science in Sports*, Epub
425 ahead of print. doi: 10.1111/sms.12501

426 Rubinstein, I., Chandilawa, R., Dagar, S., Hong, D., & Gao, X. P. (2001). Adenosine A(1)
427 receptors mediate plasma exudation from the oral mucosa. *Journal of Applied Physiology*, *91*,
428 552-60.

429 Silva-Cavalcante, M. D., Correia-Oliveira, C. R., Santos, R. A., Lopes-Silva, J. P., Lima, H.
430 M., Bertuzzi, R., ... Lima-Silva, A. E. (2013). Caffeine increases anaerobic work and restores
431 cycling performance following a protocol designed to lower endogenous carbohydrate
432 availability. *PLoS One*, *8*, e72025. doi: 10.1371/journal.pone.0072025

433 Spriet, L. L. (2014). New insights into the interaction of carbohydrate and fat metabolism
434 during exercise. *Sports Medicine*, *44*, Suppl 1, S87-96. doi: 10.1007/s40279-014-0154-1

435 Stellingwerff, T. & Cox, G. R. (2014). Systematic review: Carbohydrate supplementation on
436 exercise performance or capacity of varying durations. *Applied Physiology, Nutrition, and*
437 *Metabolism*, *39*, 998-1011. doi: 10.1139/apnm-2014-0027

438 Taylor, C., Bartlett, J. D., van de Graaf, C. S., Louhelainen, J., Coyne, V., Iqbal, Z., ...
439 Morton, J. P. (2013). Protein ingestion does not impair exercise-induced AMPK signalling
440 when in a glycogen-depleted state: implications for train-low compete-high. *European*
441 *Journal of Applied Physiology*, *113*, 1457-68. doi: 10.1007/s00421-012-2574-7

442 Wilson, P. B. (2015). Multiple Transportable Carbohydrates During Exercise: Current
443 Limitations and Directions for Future Research. *Journal of Strength and Conditioning*
444 *research*, *29*, 2056-70. doi: 10.1519/JSC.0000000000000835

445

446

447

448 **Figure Captions**

449 **Figure 1:** Schematic representation of experimental methodology. CON = control, PLA =
450 glycogen depletion and placebo mouth rinse, CAF = glycogen depletion and caffeine mouth
451 rinse.

452

453

454 **Figure 2:** Power profiles and ratings of perceived pain for five, 6 s sprints separated by 24 s
455 active rest in control (CON), glycogen depletion and placebo (PLA), and glycogen depletion
456 and caffeine (CAF) conditions. A) Peak power; B) Mean power; C) Perceived pain. Data are
457 presented as mean \pm SD. § = CON significantly greater than PLA (P<0.05). * = CON
458 significantly greater than CAF (P<0.05). # = CAF significantly greater than PLA (P<0.05).
459 ¥ = CON significantly less than PLA (P<0.05). & = CAF significantly less than PLA
460 (P<0.05).