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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

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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  $23 \pm 2$   
15 yr, body mass  $84 \pm 4$  kg, stature  $178 \pm 7$  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  $760 \pm 77$  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  $573 \pm 79$  W [ $P < 0.05$ ;  $ES = 0.850$ ]).  
27 Additionally, CAF mean power (overall mean  $589 \pm 80$  W) was significantly greater than  
28 placebo ( $519 \pm 82$  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  $581 \pm 7$  to  $533 \pm 7$  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  $53 \text{ W}$  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<sup>-1</sup>). 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<sup>-1</sup>]) 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  $760 \pm 77$  W; 95%  
197  $CI = 712-808$  W) and mean power output (overall mean  $699 \pm 83$  W; 95%  $CI = 640-758$  W)  
198 over the five sprints. There was an improvement in peak power (overall mean  $573 \pm 79$  W;  
199 95%  $CI = 516-631$  W and  $643 \pm 79$  W; 95%  $CI = 582-705$  W for PLA and CAF respectively)  
200 and mean power (overall mean  $519 \pm 82$  W; 95%  $CI = 450-578$  W and  $589 \pm 80$  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  $4 \pm 1$ ) over the five sprints. There was a significant increase in perceived pain  
204 following depletion and PLA compared to depletion and CAF ( $8 \pm 1$  and  $7 \pm 1$  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 ( $828 \pm 51$  W) was significantly greater than CAF  
212 ( $615 \pm 79$  W;  $P < 0.001$ ;  $ES = 0.850$ ) and PLA ( $627 \pm 68$  W;  $P < 0.001$ ;  $ES = 0.859$ ). During  
213 sprint two, CON peak power output ( $803 \pm 63$  W) was significantly greater than CAF ( $617 \pm$   
214  $93$  W;  $P = 0.018$ ;  $ES = 0.763$ ) and PLA ( $609 \pm 65$  W;  $P = 0.004$ ;  $ES = 0.836$ ). During sprint three,  
215 CON peak power output ( $744 \pm 73$  W) was greater than CAF ( $631 \pm 83$  W;  $P = 0.018$ ;  
216  $ES = 0.583$ ) and PLA, ( $573 \pm 71$  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, ( $542 \pm 76$  W;  $P = 0.004$ ;  $ES = 0.802$ ), but not CAF ( $654 \pm 71$   
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 ( $697 \pm 63$  W) was significantly  
221 greater than PLA, ( $518 \pm 74$  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 ( $757 \pm 72$  W) was significantly greater  
226 than CAF ( $575 \pm 82$  W) and PLA ( $[578 \pm 68$  W]  $P = 0.001$ ;  $ES = 0.765-0.789$ ). During sprint  
227 two, CON mean power output ( $740 \pm 84$  W) was significantly greater than CAF ( $576 \pm 90$  W;  
228  $P = 0.004$ ;  $ES = 0.709$ ) and PLA ( $561 \pm 71$  W;  $P = 0.002$ ;  $ES = 0.780$ ). During sprint three, CON  
229 mean power output ( $694 \pm 75$  W) was greater than CAF ( $583 \pm 87$  W;  $P = 0.014$ ;  $ES = 0.561$ )

230 and PLA, ( $510 \pm 85$  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 ( $665 \pm 62$  W)  
232 and was greater than PLA, ( $483 \pm 76$  W;  $P=0.002$ ;  $ES=0.794$ ), whilst CAF mean power  
233 output ( $596 \pm 79$  W) was also greater than PLA ( $P<0.001$ ;  $ES=0.587$ ). During sprint five,  
234 CON mean power output ( $639 \pm 75$  W) was significantly greater than PLA, ( $461 \pm 58$  W;  
235  $P=0.003$ ;  $ES=0.798$ ), whilst CAF mean power output ( $617 \pm 75$  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 ( $2 \pm 1$ ) was significantly less than CAF ( $5 \pm$   
242  $1$ ;  $P=0.001$ ;  $ES=0.853$ ) and PLA ( $6 \pm 1$   $P=0.001$ ;  $ES=0.895$ ). During sprint two, CON  
243 perceived pain ( $3 \pm 1$ ) was less than CAF ( $6 \pm 1$ ;  $P=0.001$ ;  $ES=0.853$ ) and PLA ( $7 \pm 2$ ;  
244  $P=0.001$ ;  $ES=0.896$ ). During sprint three, CON perceived pain ( $4 \pm 1$ ) was less than CAF ( $7$   
245  $\pm 1$ ;  $P=0.008$ ;  $ES=0.808$ ) and PLA, ( $8 \pm 1$ ;  $P=0.001$ ;  $ES=0.932$ ). During sprint four, CON  
246 perceived pain ( $4 \pm 1$ ) was less than CAF ( $7 \pm 2$  W;  $P=0.003$ ;  $ES=0.808$ ), and PLA, ( $9 \pm 2$ ;  
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 ( $5 \pm 1$ ) was significantly less than CAF ( $8 \pm 2$  W;  $P=0.002$ ;  
249  $ES=0.855$ ), and PLA, ( $9 \pm 1$ ;  $P<0.001$ ;  $ES=0.999$ ), whilst CAF perceived pain ( $8 \pm 1$ ) 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 ( $65 \pm 26$  min compared to  $52 \pm 23$  min). Moreover, both these  
279 conditions were superior to placebo ( $36 \pm 22$  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

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321

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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.

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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).