ORIGINAL INVESTIGATION

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

Running head: Caffeine rinsing following glycogen depletion

Abstract

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

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

Introduction

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

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

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

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

Materials and methods

Subjects

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

Design

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

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

Incremental Test

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

Carbohydrate Availability Lowering Protocol

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

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

Dietary Control

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

Repeated Sprint Cycling Test

During morning visits, participants performed five, 6 s cycling sprints under the following conditions: 1) 12–14 h after a validated exercise-protocol designed to reduce endogenous CHO availability, followed by placebo (PLA) mouth rinsing, 2) 12–14 h after a validated exercise-protocol designed to reduce endogenous CHO availability, followed by CAF mouth rinsing, and 3) with no prior depletion or mouth rinse (CON). Randomization was ensured by assigning each condition a number (1-3), then generating eight sets (one per 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 $mg \cdot kg^{-1}$) 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*

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

Results

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

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

Peak Power Output

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

Mean Power Output

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

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

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

Rating of perceived pain

During sprint one, CON perceived pain (2 ± 1) was significantly less than CAF (5 ± 1; P=0.001; ES=0.853) and PLA (6 ± 1 P=0.001; ES=0.895). During sprint two, CON perceived pain (3 ± 1) was less than CAF (6 ± 1; P=0.001; ES=0. 853) and PLA (7 ± 2; P=0.001; ES=0. 896). During sprint three, CON perceived pain (4 ± 1) was less than CAF (7 ± 1; P=0.008; ES=0.808) and PLA, (8 ± 1; P=0.001; ES=0.932). During sprint four, CON perceived pain (4 ± 1) was less than CAF (7 ± 2 W; P=0.003; ES=0.808), and PLA, (9 ± 2; P<0.001; ES=0.932), whilst CAF was less than PLA (P=0.043; ES=0.578). During sprint five, CON perceived pain (5 ± 1) was significantly less than CAF (8 ± 2 W; P=0.002; ES=0.855), and PLA, (9 ± 1; P<0.001; ES=0.999), whilst CAF perceived pain (8 ± 1) was significantly less than PLA (P=0.008; ES=0.688).

Discussion

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

Results reported here are in line with previous investigations suggesting that a) CAF mouth rinsing can improve repeated sprint exercise performance (Beaven et al., 2013), and b) CAF can reduce deleterious performance effects of glycogen depletion (Silva-Cavalcante et al., 2013; Kasper et al., 2015). Beaven et al. (2013) recently reported than when compared to placebo, CAF mouth rinsing improved peak and mean cycling power during sprint one and two (of five), yet reduced mean power during the final sprint. These authors suggested a role for caffeine in activating a supraspinal or central mechanism, capable of enhancing neural drive to motor units, accessing muscle recruitment reserve. As such, this additional muscle recruitment may have led to rapid depletion of ATP, evidenced by a reduction in mean power during the final sprint. Although our data agree, in part, with Beaven and colleagues (2013) in reporting increased peak and mean power following CAF mouth rinsing, no fatiguing effect was observed as a result of increased power profiles. Therefore, we attribute this phenomenon to the influence of glycogen depletion in the present investigation. i.e. low endogenous CHO availability did not permit recruitment of the muscle recruitment reserve. In support, Kasper et al. (2015) previously observed that the addition of a 200 mg CAF dose 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 $mg \cdot kg^{-1}$ 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 $mg \cdot kg^{-1}$ CAF increased maximal voluntary torque and muscle activation

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

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

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

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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 ± 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 ($P<0.05$).