Science and Medicine in Football

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/rsmf20

No Independent or Synergistic Effects of Carbohydrate-Caffeine Mouth Rinse on Repeated Sprint Performance During Simulated Soccer Match Play in Male Recreational Soccer Players

Lewis A. Gough, Mark Faghy, Neil Clarke, Adam L. Kelly, Matthew Cole & Wee Lun Foo

To cite this article: Lewis A. Gough, Mark Faghy, Neil Clarke, Adam L. Kelly, Matthew Cole & Wee Lun Foo (2021): No Independent or Synergistic Effects of Carbohydrate-Caffeine Mouth Rinse on Repeated Sprint Performance During Simulated Soccer Match Play in Male Recreational Soccer Players, Science and Medicine in Football, DOI: 10.1080/24733938.2021.2021277

To link to this article: https://doi.org/10.1080/24733938.2021.2021277

Accepted author version posted online: 18 Dec 2021.

Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Science and Medicine in Football*

DOI: 10.1080/24733938.2021.2021277

Title: No Independent or Synergistic Effects of Carbohydrate-Caffeine Mouth Rinse on Repeated Sprint Performance During Simulated Soccer Match Play in Male Recreational Soccer Players

Authors: Lewis A. Gough[1]., Mark Faghy[2]., Neil Clarke[3]., Adam L. Kelly[1]., Matthew Cole[1] and Wee Lun Foo[1]

Affiliations: [1]Human Performance and Health Research Group, Centre for Life and Sport Sciences (CLaSS), Birmingham City University, Birmingham, United Kingdom, B15 3TN

[2]Human Science Research Centre, University of Derby, Derby, United Kingdom, DE22 1ZS

[3]School of Life Sciences, Faculty of Health and Life Sciences, Coventry University, United Kingdom, CV1 2DS

Running Head: Mouth rinse and simulated soccer performance

Email address and contact details of corresponding author: [1]Human Performance and Health Research Group, Centre for Life and Sport Sciences (CLaSS), Birmingham City University, Birmingham, United Kingdom, B15 3TN (lewis.gough@bcu.ac.uk)

Word count: 3241

Data availability statement: The data that support the findings of this study are available on request from the corresponding author [LAG]. The data are not publicly available due to information that could compromise the privacy of research participants.

ABSTRACT

The study examined the synergistic and independent effects of carbohydrate-caffeine mouth rinse on repeated sprint performance during simulated soccer match play. Nine male soccer players (21 ± 3 years, 1.75 ± 0.05 m, 68.0 ± 9.0 kg) completed four trials with either 6 $mg \cdot kg^{-1}$ caffeine + 10% maltodextrin (CHO+CAFMR), 6 $mg \cdot kg^{-1}$ caffeine (CAFMR), 10% maltodextrin (CHOMR), water (PLA) in a block randomised, double-blinded, counterbalanced and crossover manner separated by minimum 96 h. All solutions were taste-matched and a carbohydrate-rich meal (2 $g \cdot kg^{-1}$body mass) was provided a minimum 2 h before each trial. Each trial consisted of a 90-min soccer specific aerobic field test ($SAFT^{90}$) and two bouts of repeated sprint ability tests (RSAT; 6 x 6 s sprints with 24 s recovery) completed at 0 min and 75^{th} min of $SAFT^{90}$. A 25 ml solution of either CHO+CAFMR, CAFMR, CHOMR or PLA was rinsed immediately before the second RSAT (75 min). Mean power output, peak power output (PPO) or fatigue index (FI) was not impacted by any treatment during the 75^{th} min RAST ($p > 0.05$). These results suggest that carbohydrate and/or caffeine mouth rinses do not have an ergogenic effect during simulated soccer exercise after a high carbohydrate meal.

Key words: mouth-rinse, running, performance, ergogenic aids, team sports

INTRODUCTION

Over the past 15 years mouth rinsing has been suggested to mitigate fatigue during a range of activities, including endurance (Carter et al., 2004), intermittent (Rollo et al., 2015)and strength-based exercises (Decimoni et al., 2018). Specifically, mouth rinsing with carbohydrate (CHOMR) is suggested to exert its ergogenic effect via activation of the brain regions associated with reward and pleasure (anterior cingulate cortex and ventral striatum; Chambers et al., 2009), motor output (sensorimotor cortex) and visual cue perception (intracalcarine and temporal occipital fusiform cortices; Turner et al., 2014)). Signaling pathways are also activated by CHOMR, which in turn, are suggested to enhance corticomotor output (facial, glossopharyngeal and vagus afferent pathways; Gant et al., 2010). The beneficial effect of CHOMR was first reported on 1 h cycling time trial (TT) performance (Carter et al., 2004). Since then, its effect has been explored on other types of exercises including continuous and intermittent running (Rollo et al., 2008, 2015), single and repeated sprints (Chong et al., 2011) and soccer specific performance (Matsumoto, 2013)(Arlai & Nana, 2019). The results from these studies have revealed equivocal findings, which questions the use of CHOMR on soccer performance.

Caffeine supplementation has been shown to reduce the perception of fatigue, increase jump height and enhance repeated sprint ability of soccer players (Mielgo-Ayuso et al., 2019). Soccer players are therefore recommended to consume 3-6 $mg.kg^{-1}$ body mass (BM) caffeine 60 min prior to a match (Collins et al., 2020). This strategy, however, could result in adverse effects such as anxiety, nervousness, gastrointestinal (GI) discomfort and reductions in post-exercise sleep quality and quantity (Pallarés et al., 2013; Ramos-Campo et al., 2019; Ruiz-Moreno et al., 2020). Light-caffeine-consuming athletes may experience these side effects, even with ingestion of small caffeine doses (3 $mg.kg^{-1}$) (Salinero et al., 2014). Alternatively, caffeine mouth rinse (CAFMR) can be used as a method to obtain benefits of caffeine on soccer performance while alleviate the negative connotations of ingesting a full bolus of caffeine on performance and recovery (Ehlert et al., 2020), whereby the potential performance benefits of CAFMR are expected immediately following 5-20s of mouth rinsing (Wickham & Spriet, 2018). The use of CAFMR have been shown to improve cognitive performance (De Pauw et al., 2015; Pomportes et al., 2017) and high intensity repeated cycling sprints (Kizzi et al., 2016), however, there are some conflicting findings for

resistance exercise performance (Clarke et al., 2015) and aerobic-based performance (Dolan et al., 2017; Fell et al., 2014; Sinclair & Bottoms, 2014). Nonetheless, if CAFMR was to be ergogenic in football this would particularly benefit evening games by leading to better sleep quality and quantity and for a subset of players who are more susceptible to adverse effects of caffeine. Whilst it was initially suggested CAFMR can lead to blood stream absorption (through increased permeability of the buccal mucosa), a more feasible mechanism could be that CAFMR triggers the bitter taste receptors (Matsumoto, 2013) and subsequently activate the brain regions associated with information processing and reward (for review, see Wickham & Spriet, 2018).

Due to the distinct mechanisms between CHOMR and CAFMR, a combination of both rinses (CHO+CAFMR) had been found to have greater impact on repeated sprint and cognitive performance compared to separate use of both substances (Beaven et al., 2013; Meeusen et al., 2017). Nonetheless, the mouth rinsing of CHO+CAFMR has been underexplored to date in soccer related performance, with only two studies reporting non-significant improvement in technical soccer skills (Arlai & Nana, 2019) and intermittent running (Dolan et al., 2017). These findings may be related to the short duration of exercise protocol used (≤45 min) as positive effect of mouth rinsing was reported in the final 15 min of a 90 min soccer simulated trial (Rollo et al., 2015). However, in the latter study, the pre-trial meal was not standardised and the dietary intake was not recorded prior to each trial. The impact of this could explain the high inter-individual variability in sprint times following CHOMR the authors reported. Moreover, limited research has explored the impact of a standardised meal on the subsequent impact of carbohydrate and caffeine mouth rinsing (CHO+CAFMR). This is an important factor to consider for future research considering the smaller improvement in performance of CHOMR in a fed state than a fasted state (Fares & Kayser, 2011; Lane et al., 2013). Equally, as soccer players typically ingest a high carbohydrate meal before training or competitive games, it is important these practices be followed when investigating the efficacy of mouth rinses (Oliveira et al., 2017). Therefore, the aim of this study was to evaluate the synergistic or independent effects of CHO+CAFMR on repeated sprint ability among recreational soccer players following ingestion of a high CHO meal.

METHODS

Participants

Statistical power analysis performed with the G*Power 3 program (Faul et al., 2007) using the effect size of 0.81 from Beaven et al. (2013) revealed a required sample size of eight participants at an alpha value of 0.05 and a power of 0.8. Nine male recreational soccer players (age: 21 ± 3 years, height: 1.75 ± 0.05 m, body mass: 68.0 ± 9.0 kg, body fat: 14.7 ± 7.1 %) with 12 ± 4 years of experience (non-professional) gave their written informed consent before participating in this study approved by an Ethical Advisory Committee. Daily caffeine consumption was collected via dietary recall (daily caffeine consumption: 1.0 ± 1.1 $mg \cdot kg^{-1} \cdot day^{-1}$; Naive consumer (<25 $mg.day^{-1}$, *n* = 3); Low consumer (25 $mg.day^{-1}$ to 0.99 $mg \cdot kg^{-1} \cdot day^{-1}$, *n* =3); Mild consumer (1.00-2.99 $mg \cdot kg^{-1} \cdot day^{-1}$, n=2); Moderate consumer (3.00-5.99 $mg \cdot kg^{-1} \cdot day^{-1}$, n=1) (Filip et al., 2020). This research was conducted in accordance with the Declaration of Helsinki.

Study Design

The study employed a double-blinded counterbalanced, crossover design. Each participant completed five trials at a similar time (±2 h), separated by at least 96 h. Participants were initially required to complete a Physical Activity Readiness Questionnaire (PAR-Q) and self-reported their daily caffeine intake (through dietary recall). During the first visit participants' height, weight and body composition were measured using a stadiometer (SEC-225, Seca, Hamburg, Germany), digital scale (SEC-170, Seca, Hamburg, Germany) and Bioelectrical Impedance Analysers (KaradaScan Body Compostion Analyser, Omron). Participants underwent a familiarisation trial which comprised of one 15 min section of $SAFT^{90}$ (Small et al., 2010) and one set of a modified repeated sprint ability test (RSAT; Rampinini et al., 2007) that comprised of 6 x 6s sprints with 24s recovery on a non-motorised treadmill (SkillMill™ Connect, Technogym, Italy). Participants were then introduced to The Feeling Scale (FS; Hardy & Rejeski, 1989), The Felt Arousal Scale (FAS; Svebak & Murgatroyd, 1985), The Rating of Perceived Exertion Scale (RPE; Borg, 1982) and the mouth rinsing protocol (water; PLA). Participants were requested to abstain from vigorous exercise and alcohol 24 h before and caffeine 12 h before visits 2-5. Participants were also asked to record their dietary intake 24 h before visit 2 and replicate it before visits 3-5. Participants were

instructed to consume the high CHO meal ($2g \cdot kg^{-1}$ BM) provided beforehand that includes 500ml of fluid (250ml milk and 250ml of fruit juice) unsupervised 2 h ± 10 min prior to experimental trials to ensure adequate muscle glycogen (Oliveira et al., 2017) and hydration status before each trial. The pre-exercise meal was standardised for energy and macronutrient composition, and the researcher informed the participant of the amount of intake that was required. To confirm adherence, participants sent photographic evidence both pre, and post-meal to the researcher.

Experimental Procedures

On visit 2-5, participants completed the experimental protocol shown in Figure 1 in a temperature controlled indoor sports hall. During each visit, a full $SAFT^{90}$ protocol and two bouts of RSAT at 0 and 75^{th} min of $SAFT^{90}$ were completed by the participants. Participants completed their self-instructed warm up including jogging, striding and stretching prior to first RSAT, and this was replicated for each trial. Only ad libitum plain water intake was allowed during the experimental trial. Peak power output (PPO), mean power output (MPO) and fatigue index (FI) were recorded from each RSAT. FI was calculated via the following equation: [(PPO (W) – Lowest Power Output (W))/ PPO (W)] × 100. This equation was selected as it was previously used to measure FI of RSAT in Division I soccer players (Sanders et al., 2017). Capillary blood samples were taken at baseline and during 2 min recovery after 2^{nd} RSAT via finger prick for measurement of blood glucose and blood lactate (Biosen C-Line, EKF Diagnostics, Germany). FS and FAS were administered at rest and at every 15 min throughout $SAFT^{90}$ while RPE was administered at 15 min intervals during $SAFT^{90}$.

Exercise Protocol

The design of $SAFT^{90}$ requires participants to shuttle run over a 20 m distance, with four poles positioned for participants to navigate with utility movements. Participants either backwards run or sidestep around the first pole, followed by forwards run through the course, navigating the middle three poles. An audio CD was played to provide verbal signals to maintain the exercise intensity and activity performed by the participants during $SAFT^{90}$. A 15 min activity profile was repeated six times during the full 90 min simulated soccer match. Over the 90 min, participants completed 1269 changes in speed and 1350 changes in

direction, although participants did not perform other soccer specific actions such as kicking, dribbling, tackling and heading.

Supplementation protocol

Mouth rinse solutions (25ml) were provided in a non-transparent plastic cup. Participants were asked to rinse the solutions for 10s (Sinclair et al., 2014) before commencing the 2nd RSAT at 75th min of SAFT^{90} and expectorate the solutions into the pre-weighed cup for post measurement. The solutions were as followed: 1) 10% Maltodextrin (100% Maltodextrin Carbs, MyProtein, UK) (CHOMR), 2) 6mg·kg^{-1} BM caffeine (Caffeine Powder, Bulk Powders, UK) (CAFMR), 3) 10% Maltodextrin + 6mg·kg^{-1} BM Caffeine (CHO+CAFMR) and 4) taste-matched control (PLA). The caffeine dose 6mg·kg^{-1} BM caffeine was selected for CAFMR as CAFMR with this dose was shown to be ergogenic for repeated cycling performance (Kizzi et al., 2016). Supplement order was randomised using a block randomised method (n = 9, 1 block, 4 treatments, source: www.randomisation.com) by an individual not involved in the study. Non-calorific artificial sweetener consists of sucralose (FlavDrops, MyProtein, UK) were added to each solution to make the solutions indistinguishable. A laboratory technician who was not involved in the study prepared solutions. Supplementation order was only revealed to participants and researchers at the end of the study.

Statistical Analysis

Normality of all data was verified by using visual inspection of Q-Q plot and Histogram, Shapiro-Wilk Statistics and z scores of skewness and kurtosis. Data was analysed using a two-way (treatment x time) repeated measures ANOVA for performance (MPO, PPO and FI), perceptual measures (FS, FAS, and RPE), and blood measures (blood glucose and lactate). A Bonferroni adjusted post hoc test was used to locate variance, where significant statistical effects occurred. Where main effects or interactions were observed, partial eta squared ($P\eta^2$) effect size was reported. $P\eta^2$ was interpreted as small (0.01), medium (0.06) and large (0.14) (Cohen, 1988). Pairwise effect size comparisons were calculated using Cohens d and interpreted as small (0.2) moderate (0.5) or large (0.8) (Cohen, 1988). Using a function of P-value, F-value and degrees of freedom generated by ANOVA, the effect of interaction was expressed as 95% confidence interval (CI) of whether the true effect indicated a positive, negative or trivial change in performance (A. M. Batterham & Hopkins, 2006). An

effect was deemed unclear if the confidence intervals span both positive and negative thresholds, whilst those that did not cross the zero boundary were deemed significant. To identify individual differences in mean power output from the 2nd RSAT, the Smallest Worthwhile Change (SWC) statistic was used (0.3*SD) (Hopkins, 2004). Statistical significance was set as $p < 0.05$. Statistical analyses were performed by using SPSS 25.0 software (Chicago, IL, USA).

RESULTS

Mean power output was not impacted by any treatment (all $p > 0.05$; Figure 2A). There was a large inter-individual variation in MPO between treatments (Table 1). Equally, no impact on PPO ($p=0.199$, $P\eta^2 = 0.173$) or FI ($p=0.726$, $P\eta^2 = 0.052$) was observed between treatments. All pairwise effect size comparisons for MPO, PPO and FI were less than $g < 0.20$.

No treatment had an impact on FAS ($p=0.568$, $P\eta^2 = 0.087$), FS ($p=0.441$, $P\eta^2 = 0.109$), or RPE ($p=0.171$, $P\eta^2 = 0.167$) (Table 2). The mean volume of expectorate for the CHO+CAFMR, CAFMR, CHOMR and PLA trials were 25±1 ml, 24±2 ml, 25±0 ml and 24±1 ml respectively.

No treatment had an impact on either blood glucose ($p=0.716$, $P\eta^2 = 0.054$; Figure 3A), or blood lactate concentrations ($p = 0.864$, $P\eta^2 = 0.030$; Figure 3B). A main effect of time ($p<0.001$, $P\eta^2 = 0.880$) with blood lactate concentrations between baseline (1.7 ± 0.2 mmol.l^{-1}; 95% CI = 1.3 to 2.0) and 2nd RSAT (7.3 ± 0.8 mmol.l^{-1}; 95% CI = 5.6 to 9.1) were found.

DISCUSSION

The primary aim of this study was to investigate the effect of mouth rinsing with either CHOMR, CAFMR or a combination (CHO+CAFMR) on repeated sprint performance among male recreational soccer players. This is the first study to compare a combination strategy (CHO+CAFMR) to individual rinses (CHOMR and CAFMR) during 90 min simulated soccer performance following a standardised pre-exercise high carbohydrate meal. The results from the study suggest none of the mouth rinses used in this study influence

physiological (blood glucose and lactate), perceptual (FAS, RPE) or performance (MPO, PPO, FI) during a simulated soccer protocol. A small number of participants (n = 2) did improve performance in the either CHOMR or CAFMR treatments however, which suggests that mouth rinsing strategies should be tested on and individual bases. Nonetheless, as most of the participants reported no ergogenic benefits from any mouth rinse, it is unlikely individuals will obtain a performance benefit from the mouth rinsing strategies used in this study.

The lack of ergogenic benefit observed from the combination of CHO+CAFMR is contradictory to a previous study reporting ergogenic benefits (Beaven et al., 2013). The authors reported an increase in MPO compared to the CHOMR only trial during sprint 5 of cycling RAST (5 x 6 s sprints; 24 s recovery) (mean difference = 22.1 ± 28.3 W; ES = 0.66). Most studies employing a RAST test using cycling report ergogenic effects following either CAFMR or CHOMR (or in combination) mouth rinse (Beaven et al., 2013), whilst those more akin to the current study using a longer duration exercise and a running modality report non-significant finding (Dorling & Earnest, 2013). The reasons for this discrepancy are ambiguous, although it could be speculated that longer duration protocols are reliant upon glycogen depletion, and the protocols used to date have not caused this (Dorling & Earnest, 2013; present study). Whereas during the shorter duration protocols used by Beavan et al. (2013) mouth rinsing might be able to stimulate the brain regions associated with reward and pleasure, inferring a central mechanism that led to ergogenic benefits (Chambers et al., 2009; Matsumoto, 2013). Conversely, Tomazin et al., (2017) showed that central fatigue was only evident in running RAST and not cycling, which contradicts the suggestion of the current study data. It is worth noting that other discrepancies such as the training status of participants, pre-experimental controls on nutrition, and dosing patterns were evidence between studies to date, therefore further research is required to pinpoint the precise mechanisms of action.

Despite finding no significant effect on MPO for any mouth rinse treatments in the present study, there was a number of individuals who improved in one or more mouth rinse treatments. Specifically, participants seven and eight improved in two treatments versus the placebo (CAFMR and CHOMR). These findings partially support the inter-individual

variation findings of Rollo et al. (2015) who also reported three individuals performances were reduced by a CHOMR compared to a placebo, whilst the other eight improved. The present study findings only partially agree, however, as the most of our participants did not improve performance following any mouth rinse (6/9). Moreover, Rollo et al. (2015) was a CHOMR study, therefore the present study adds that CHOMR, CAFMR or CHO+CAFMR will be ineffective for most individuals. Interestingly, those that improved with CHOMR or CAFMR did not improve with CHO+CAFMR, which is in contrast to Beaven et al. (2013) who reported that CHO+CAFMR provided additional significant performance benefits over CHOMR. This was despite the present study using a higher dose of both ingredients (CHO: 10% vs. 6%; CAF: 2% vs. 1.2%). Based on the inter-individual differences in the present study, it is recommended that the use of mouth rinsing is not generalised to a population and instead should be trialled on an individual basis. Furthermore, dose-response studies are required to determine the optimal dosages for CHO+CAFMR that will ensure an additional ergogenic effect over CHOMR and CAFMR.

It is plausible that the pre-experimental dietary status in the current study, through being in a fed state, negatively affected the mechanism of action associated with mouth rinsing. Indeed, Haase et al., (2009) reported following the ingestion of a 700 kcal liquid meal (FAT = 22 g, CHO = 94 g, protein = 32 g) after an overnight fast resulted in lower activation in reward-related brain regions in response to oral sucrose (CHO) and caffeine when compared to a fasted condition. This might be related to the modulation of brain regions by the homeostatic signals including peptide YY (R. L. Batterham et al., 2007), ghrelin (Malik et al., 2008) and leptin (Farooqi et al., 2007) following food intake. Consequently, the absence of an ergogenic effects from any of the mouth rinses used in the present study could be attribute to this change in brain responses, as a high calorie CHO meal was ingested shortly before the $SAFT^{90}$. These findings also corroborate with other studies showing that participants in a fed state do no improve performance following mouth rinse, or the performance improvement is dampened compared to fasted states (Fares & Kayser, 2011; Lane et al., 2013). This claim should be interpreted with caution, however, as this study did not examine brain responses other than perceptual feelings of RPE, FAS and FS. Importantly, the current study did not find any significant changes in any of these perceptual brain-linked responses for any mouth rinse, which previous studies corroborate (Rollo et al., 2008; Rollo et al., 2010; Carter et al., 2004). The lack of difference observed in the present study could be attributed to the near-

maximal RPE values elicited by RSAT, and as a result, this created a 'ceiling effect' that makes any significant difference between treatments hard to distinguish (Beaven et al., 2013).

Our participants were instructed to mouth rinse the allocated solutions for 10s as 10s CHOMR was superior to 5s CHOMR in improving cycling time trial performance (Sinclair et al., 2014). Additionally, CAFMR and CHO+CAFMR for 5-10s have also been shown to be beneficial on repeated sprint cycling performance (Beaven et al., 2013; Kizzi et al., 2016). Interestingly, some studies have shown positive effects of CAFMR with a longer mouth rinse duration (20s), although a dose response to the duration of mouth rinse was not examined (De Pauw et al., 2015; Pomportes et al., 2017). It is therefore plausible that longer mouth rinse may be required to achieve ergogenic effects in the current study given that the experiments were performed under a fed state whereby the brain activation is dampened, Our data, however, cannot confirm this hypothesis and hence further research is warranted.

A limitation of this study was that it was impossible to taste-match the solutions due to the strong bitter taste of caffeine (despite added artificial sweetener) and it is therefore unclear if this could have affected participants' performance due to detecting the supplement. A supplement detection questionnaire would have offered insight here; therefore, we recommend that this procedure be used in future research to appropriately assess the efficacy of blinding procedures. Furthermore, participants only performed one familiarisation trial for RSAT, which may not be sufficient to exclude the learning effect. Our study, however, adopted a randomised counterbalanced design as recommended by Brooks (2012) and hence a trial-order effect was prevented (Table 3). Moreover, we also acknowledge that our findings are relevant to a single mouth rinse and in conditions where the participants did not ingest anything at half time. Some, but not all, individuals will consume CHO during half time, which could affect the efficacy of the mouth rinse(s), although previous research has shown many professional players ingest inadequate CHO intake (Anderson et al., 2017). Lastly, the low number and single sex status of participants in this study was a limitation, combined with the fact they were not professional soccer players. Future research could therefore investigate individuals of a higher training status and recruit more participants to make the findings more ecologically valid, however, it is acknowledged how difficult this can be in practice.

In conclusion, results from the present study indicate that repeated sprint performance during simulated soccer match play was not significantly improved following a mouth rinse with

either CHOMR or CAFMR in isolation or in combination. It is likely any potential ergogenic benefits were blocked by the ecologically valid pre-experimental dietary practice of consuming a high carbohydrate meal, although future research should attempt to directly assess glycogen depletion. Our results suggest practitioners and athletes are not required to consider mouth rinsing if a sufficient pre-exercise meal has been consumed.

Acknowledgements: The authors would like to thank the lab technician for conducting the double blinding for the solutions and counterbalance of the participants' order. The authors acknowledge the hard work and commitment of the participants who gave their time to participate in this research project.

Authorship: LG, MC, WF designed the study. WF completed the data collection for the study. LG, WF, MC, AK, MF, NC contributed to the writing of the manuscript. All authors reviewed and approved the final version of the paper.

Conflict of interest: All authors report no conflict of interest

Funding sources: No funding was provided for this study

References

Anderson, L., Naughton, R. J., Close, G. L., Di Michele, R., Morgans, R., Drust, B., & Morton, J. P. (2017). Daily Distribution of Macronutrient Intakes of Professional Soccer Players From the English Premier League. *International Journal of Sport Nutrition and Exercise Metabolism*, *27*(6), 491–498. https://doi.org/10.1123/ijsnem.2016-0265

Arlai, S. and Nana, A. (2019) Carbohydrate and caffeine mouth rinse after 45-min of an intermittent shuttle run test did not enhance soccer performance in collegiate players. *Journal of Sports Science and Technology*, pp.37-48.

Batterham, A. M., & Hopkins, W. G. (2006). Making meaningful inferences about magnitudes. *International Journal of Sports Physiology and Performance*, *1*(1), 50–57. https://doi.org/10.1123/ijspp.1.1.50

Batterham, R. L., ffytche, D. H., Rosenthal, J. M., Zelaya, F. O., Barker, G. J., Withers, D. J., & Williams, S. C. R. (2007). PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans. *Nature*, *450*(7166), 106–109. https://doi.org/10.1038/nature06212

Beaven, C. M., Maulder, P., Pooley, A., Kilduff, L., & Cook, C. (2013). Effects of caffeine and carbohydrate mouth rinses on repeated sprint performance. *Applied Physiology, Nutrition, and Metabolism*, *38*(6), 633–637. https://doi.org/10.1139/apnm-2012-0333

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise*, *14*(5), 377–381.

Brooks, J. L. (2012). Counterbalancing for serial order carryover effects in experimental condition orders. *Psychological Methods*, *17*(4), 600–614. https://doi.org/10.1037/a0029310

Carter, J. M., Jeukendrup, A. E., & Jones, D. A. (2004). The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Medicine and Science in Sports and Exercise*, *36*(12), 2107–2111. https://doi.org/10.1249/01.mss.0000147585.65709.6f

Chambers, E. S., Bridge, M. W., & Jones, D. A. (2009). Carbohydrate sensing in the human mouth: Effects on exercise performance and brain activity. *The Journal of Physiology*, *587*(Pt 8), 1779–1794. https://doi.org/10.1113/jphysiol.2008.164285

Chong, E., Guelfi, K. J., & Fournier, P. A. (2011). Effect of a carbohydrate mouth rinse on maximal sprint performance in competitive male cyclists. *Journal of Science and Medicine in Sport*, *14*(2), 162–167. https://doi.org/10.1016/j.jsams.2010.08.003

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

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*.

Collins, J., Maughan, R. J., Gleeson, M., Bilsborough, J., Jeukendrup, A., Morton, J. P., Phillips, S. M., Armstrong, L., Burke, L. M., Close, G. L., Duffield, R., Larson-Meyer, E., Louis, J., Medina, D., Meyer, F., Rollo, I., Sundgot-Borgen, J., Wall, B. T., Boullosa, B., … McCall, A. (2020). UEFA expert group statement on nutrition in elite football. Current evidence to inform practical recommendations and guide future research. *British Journal of Sports Medicine*. https://doi.org/10.1136/bjsports-2019-101961

De Pauw, K., Roelands, B., Knaepen, K., Polfliet, M., Stiens, J., & Meeusen, R. (2015). Effects of caffeine and maltodextrin mouth rinsing on P300, brain imaging, and cognitive performance. *Journal of Applied Physiology*, *118*(6), 776–782. https://doi.org/10.1152/japplphysiol.01050.2014

Decimoni, L. S., Curty, V. M., Almeida, L., Koch, A. J., Willardson, J. M., & Machado, M. (2018). Carbohydrate mouth rinsing improves resistance training session performance. *International Journal of Sports Science & Coaching*, *13*(5), 804–809. https://doi.org/10.1177/1747954118755640

Dolan, P., Witherbee, K. E., Peterson, K. M., & Kerksick, C. M. (2017). Effect of Carbohydrate, Caffeine, and Carbohydrate + Caffeine Mouth Rinsing on Intermittent

Running Performance in Collegiate Male Lacrosse Athletes. *Journal of Strength and Conditioning Research*, *31*(9), 2473–2479. https://doi.org/10.1519/JSC.0000000000001819

Dorling, J. L., & Earnest, C. P. (2013). Effect of carbohydrate mouth rinsing on multiple sprint performance. *Journal of the International Society of Sports Nutrition*, *10*(1), 41. https://doi.org/10.1186/1550-2783-10-41

Ehlert, A. M., Twiddy, H. M., & Wilson, P. B. (2020). The Effects of Caffeine Mouth Rinsing on Exercise Performance: A Systematic Review. *International Journal of Sport Nutrition and Exercise Metabolism*, *30*(5), 362–373. https://doi.org/10.1123/ijsnem.2020-0083

Fares, E.-J. M., & Kayser, B. (2011). Carbohydrate Mouth Rinse Effects on Exercise Capacity in Pre- and Postprandial States. *Journal of Nutrition and Metabolism*, *2011*, 385962. https://doi.org/10.1155/2011/385962

Farooqi, I. S., Bullmore, E., Keogh, J., Gillard, J., O'Rahilly, S., & Fletcher, P. C. (2007). Leptin regulates striatal regions and human eating behavior. *Science (New York, N.Y.)*, *317*(5843), 1355. https://doi.org/10.1126/science.1144599

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*(2), 175–191. https://doi.org/10.3758/bf03193146

Fell, J. W., Doering, T. M., Shing, C. M., Leveritt, M. D., & Desbrow, B. (2014). The Effect of a Caffeinated Mouth-Rinse on Endurance Cycling Time-Trial Performance. *International Journal of Sport Nutrition and Exercise Metabolism*, *24*(1), 90–97. https://doi.org/10.1123/ijsnem.2013-0103

Filip, A., Wilk, M., Krzysztofik, M., & Del Coso, J. (2020). Inconsistency in the Ergogenic Effect of Caffeine in Athletes Who Regularly Consume Caffeine: Is It Due to the Disparity in the Criteria That Defines Habitual Caffeine Intake? *Nutrients*, *12*(4), E1087. https://doi.org/10.3390/nu12041087

Gant, N., Stinear, C. M., & Byblow, W. D. (2010). Carbohydrate in the mouth immediately facilitates motor output. *Brain Research*, *1350*, 151–158. https://doi.org/10.1016/j.brainres.2010.04.004

Haase, L., Cerf-Ducastel, B., & Murphy, C. (2009). Cortical activation in response to pure taste stimuli during the physiological states of hunger and satiety. *NeuroImage*, *44*(3), 1008–1021. https://doi.org/10.1016/j.neuroimage.2008.09.044

Hardy, C. J., & Rejeski, W. J. (1989). Not What, but How One Feels: The Measurement of Affect during Exercise. *Journal of Sport and Exercise Psychology*, *11*(3), 304–317. https://doi.org/10.1123/jsep.11.3.304

Hopkins, W. G. (2004). How to interpret changes in an athletic performance test. *Sportscience*, *8*, 1-7.

Kizzi, J., Sum, A., Houston, F. E., & Hayes, L. D. (2016). Influence of a caffeine mouth rinse on sprint cycling following glycogen depletion. *European Journal of Sport Science*, *16*(8), 1087–1094. https://doi.org/10.1080/17461391.2016.1165739

Lane, S. C., Bird, S. R., Burke, L. M., & Hawley, J. A. (2013). Effect of a carbohydrate mouth rinse on simulated cycling time-trial performance commenced in a fed or fasted state. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition Et Metabolisme*, *38*(2), 134–139. https://doi.org/10.1139/apnm-2012-0300

Malik, S., McGlone, F., Bedrossian, D., & Dagher, A. (2008). Ghrelin modulates brain activity in areas that control appetitive behavior. *Cell Metabolism*, *7*(5), 400–409. https://doi.org/10.1016/j.cmet.2008.03.007

Matsumoto, I. (2013). Gustatory Neural Pathways Revealed by Genetic Tracing from Taste Receptor Cells. *Bioscience, Biotechnology, and Biochemistry*, *77*(7), 1359–1362.

Meeusen, R., Marcora, S., Roelands, B., De Pauw, K., & Van Cutsem, J. (2017). A caffeine-maltodextrin mouth rinse counters mental fatigue. *Psychopharmacology*, *235*(4), 947–958. https://doi.org/10.1007/s00213-017-4809-0

Mielgo-Ayuso, J., Calleja-Gonzalez, J., Del Coso, J., Urdampilleta, A., León-Guereño, P., & Fernández-Lázaro, D. (2019). Caffeine Supplementation and Physical Performance, Muscle Damage and Perception of Fatigue in Soccer Players: A Systematic Review. *Nutrients*, *11*(2), 440. https://doi.org/10.3390/nu11020440

Oliveira, C. C., Ferreira, D., Caetano, C., Granja, D., Pinto, R., Mendes, B., & Sousa, M. (2017). Nutrition and Supplementation in Soccer. *Sports (Basel, Switzerland)*, *5*(2), E28. https://doi.org/10.3390/sports5020028

Pallarés, J. G., Fernández-Elías, V. E., Ortega, J. F., Muñoz, G., Muñoz-Guerra, J., & Mora-Rodríguez, R. (2013). Neuromuscular responses to incremental caffeine doses: Performance and side effects. *Medicine and Science in Sports and Exercise*, *45*(11), 2184–2192. https://doi.org/10.1249/MSS.0b013e31829a6672

Pomportes, L., Brisswalter, J., Casini, L., Hays, A., & Davranche, K. (2017). Cognitive performance enhancement induced by caffeine, carbohydrate and guarana mouth rinsing during submaximal exercise. *Nutrients*, *9*(6), 1–17. https://doi.org/10.3390/nu9060589

Ramos-Campo, D. J., Pérez, A., Ávila-Gandía, V., Pérez-Piñero, S., & Rubio-Arias, J. Á. (2019). Impact of Caffeine Intake on 800-m Running Performance and Sleep Quality in Trained Runners. *Nutrients*, *11*(9), E2040. https://doi.org/10.3390/nu11092040

Rampinini, E., Bishop, D., Marcora, S. M., Ferrari Bravo, D., Sassi, R., & Impellizzeri, F. M. (2007). Validity of simple field tests as indicators of match-related physical performance in top-level professional soccer players. *International Journal of Sports Medicine*, *28*(3), 228–235. https://doi.org/10.1055/s-2006-924340

Rollo, I., Homewood, G., Williams, C., Carter, J., & Goosey-Tolfrey, V. L. (2015). The Influence of Carbohydrate Mouth Rinse on Self-Selected Intermittent Running Performance. *International Journal of Sport Nutrition and Exercise Metabolism*, *25*(6), 550–558. https://doi.org/10.1123/ijsnem.2015-0001

Rollo, I., Williams, C., Gant, N., & Nute, M. (2008). The influence of carbohydrate mouth rinse on self-selected speeds during a 30-min treadmill run. *International Journal of Sport Nutrition and Exercise Metabolism*, *18*(6), 585–600. https://doi.org/10.1123/ijsnem.18.6.585

Ruiz-Moreno, C., Lara, B., Salinero, J. J., Brito de Souza, D., Ordovás, J. M., & Del Coso, J. (2020). Time course of tolerance to adverse effects associated with the ingestion of a moderate dose of caffeine. *European Journal of Nutrition*, *59*(7), 3293–3302. https://doi.org/10.1007/s00394-019-02167-2

Salinero, J. J., Lara, B., Abian-Vicen, J., Gonzalez-Millán, C., Areces, F., Gallo-Salazar, C., Ruiz-Vicente, D., & Del Coso, J. (2014). The use of energy drinks in sport: Perceived ergogenicity and side effects in male and female athletes. *The British Journal of Nutrition*, *112*(9), 1494–1502. https://doi.org/10.1017/S0007114514002189

Sanders, G. J., Turner, Z., Boos, B., Peacock, C. A., Peveler, W., & Lipping, A. (2017). Aerobic Capacity is Related to Repeated Sprint Ability with Sprint Distances Less Than 40 Meters. *International Journal of Exercise Science*, *10*(2), 197–204.

Sinclair, J., & Bottoms, L. (2014). The Effects of Carbohydrate and Caffeine Mouth Rinsing on Arm Crank Time-Trial Performance. *Journal of Sports Research*, *1*(2), 34–44. https://doi.org/10.18488/journal.90/2014.1.2/90.2.34.44

Sinclair, J., Bottoms, L., Flynn, C., Bradley, E., Alexander, G., McCullagh, S., Finn, T., & Hurst, H. T. (2014). The effect of different durations of carbohydrate mouth rinse on cycling performance. *European Journal of Sport Science*, *14*(3), 259–264. https://doi.org/10.1080/17461391.2013.785599

Small, K., McNaughton, L., Greig, M., & Lovell, R. (2010). The effects of multidirectional soccer-specific fatigue on markers of hamstring injury risk. *Journal of Science and Medicine in Sport*, *13*(1), 120–125. https://doi.org/10.1016/j.jsams.2008.08.005

Svebak, S., & Murgatroyd, S. (1985). Metamotivational dominance: A multimethod validation of reversal theory constructs. *Journal of Personality and Social Psychology*, *48*(1), 107–116. https://doi.org/10.1037/0022-3514.48.1.107

Turner, C. E., Byblow, W. D., Stinear, C. M., & Gant, N. (2014). Carbohydrate in the mouth enhances activation of brain circuitry involved in motor performance and sensory perception. *Appetite*, *80*, 212–219. https://doi.org/10.1016/j.appet.2014.05.020

Tomazin, K., Morin, J. B., & Millet, G. Y. (2017). Etiology of neuromuscular fatigue after repeated sprints depends on exercise modality. *International journal of sports physiology and performance*, *12*(7), 878-885. https://doi.org/10.1123/ijspp.2016-0200

Wickham, K. A., & Spriet, L. L. (2018). Administration of Caffeine in Alternate Forms. *Sports Medicine (Auckland, N.Z.)*, *48*(Suppl 1), 79–91. https://doi.org/10.1007/s40279-017-0848-2

Table 1. Individual responses in MPO (watts) displaying the difference between the 1st RSAT (baseline) and the 2nd RSAT depending on treatment. Individuals above the Smallest Worthwhile Change (SWC) (six watts) versus placebo are in bold and #.

Participant number	CHO+CAFMR	CAFMR	CHOMR	PLA
1	3	8	6	12
2	-12	-17	1	22
3	-2	-25	-5	3
4	-26	-6	11	5
5	-22	4	-23	34
6	-16	3	-7	3
7	1	**19#**	**8#**	-4
8	-2	**12#**	**14#**	4
9	1	11	3	10

Table 2: Psychological scores for The Felt Arousal Scale (FAS), The Feeling Scale (FS) and The Rate of Perceived Exertion Scale (RPE) during the experimental trials. (CHO+CAF = Carbohydrate and caffeine mouth rinse; CHO = Carbohydrate mouth rinse only; CAF = Caffeine mouth rinse only; PLA = Placebo; RSAT = Repeated sprint ability test).

Measure	Rest	After 1st RSAT	15 min	30 min	45 min	60 min	75 min	After 2nd RSAT	90 min
FAS									
CHO+CAF	2.4 ± 1.1	3.1 ± 0.6	2.9 ± 0.6	3.0 ± 0.5	2.9 ± 0.3	2.9 ± 1.3	3.0 ± 1.0	2.9 ± 0.6	3.2 ± 0.8
CAF	2.3 ± 0.9	2.9 ± 0.8	3.1 ± 0.6	3.4 ± 0.9	3.1 ± 0.3	3.0 ± 0.5	3.1 ± 0.8	3.0 ± 1.1	3.2 ± 1.0
CHO	2.4 ± 0.9	3.1 ± 0.9	3.0 ± 1.0	3.1 ± 1.1	3.3 ± 0.7	3.6 ± 0.5	3.1 ± 0.8	3.3 ± 0.9	3.7 ± 0.7
PLA	2.7 ± 0.9	2.8 ± 0.7	2.8 ± 0.7	3.0 ± 0.7	3.0 ± 0.5	3.2 ± 0.7	3.1 ± 0.9	3.2 ± 1.0	3.1 ± 0.9
FS									
CHO+CAF	1.3 ± 1.4	0.6 ± 1.6	0.7 ± 1.5	0.3 ± 1.4	0.2 ± 0.8	0.6 ± 1.1	0.0 ± 0.7	-0.2 ± 1.0	0.0 ± 1.2
CAF	1.7 ± 1.3	0.8 ± 1.5	0.0 ± 1.9	-0.1 ± 1.8	0.7 ± 1.5	0.8 ± 1.2	0.1 ± 1.3	0.2 ± 1.8	0.1 ± 1.8
CHO	1.2 ± 1.3	0.1 ± 1.9	-0.2 ± 2.0	-0.4 ± 2.1	0.0 ± 1.2	0.1 ± 1.5	-0.1 ± 1.2	-0.1 ± 1.5	-0.3 ± 1.7
PLA	1.0 ± 1.1	0.7 ± 1.0	-0.3 ± 1.4	0.2 ± 0.8	-0.3 ± 1.7	0.2 ± 1.7	-0.2 ± 1.7	0.6 ± 1.5	0.4 ± 1.6
RPE									

CHO+CAF	n/a	12.8 ± 2.3	13.6 ± 1.1	13.8 ± 1.9	13.9 ± 1.9	14.2 ± 2.1	14.3 ± 2.4	15.8 ± 2.5	15.0 ± 2.3
CAF	n/a	13.9 ± 2.5	14.2 ± 1.8	14.2 ± 1.9	14.0 ± 1.4	14.2 ± 1.6	14.9 ± 2.1	15.0 ± 2.7	16.2 ± 1.6
CHO	n/a	12.9 ± 3.5	13.4 ± 1.9	14.0 ± 2.1	13.9 ± 2.0	13.8 ± 1.9	14.6 ± 2.4	15.1 ± 2.8	15.6 ± 2.3
PLA	n/a	13.1 ± 3.3	14.3 ± 1.7	14.9 ± 1.8	14.4 ± 2.2	14.4 ± 2.7	14.9 ± 2.5	14.1 ± 2.7	14.9 ± 1.7

Table 3: The trial order effects on mean power output, peak power output and fatigue index.

Variables	**Baseline**				**At 75th min**				**Interaction effect**
	1st	2nd	3rd	4th	1st	2nd	3rd	4th	
Mean Power Output (W)	213±29	221±24	226±21	225±22	215±31	226±27	223±29	225±24	(F (3, 24) = 0.673, p=0.577, $P\eta^2$ = 0.078)
Peak Power Output (W)	229±29	234±27	244±25	239±24	233±33	244±28	236±31	243±29	(F (3, 24) = 2.057, p=0.133, $P\eta^2$ = 0.205)

Fatigue Index (%)	15.40±5.54	11.63±4.44	13.62±3.91	11.22±5.29	15.24±5.82	14.06±4.89	10.99±2.71	12.77±3.47	(F (3, 24) = 1.512, p=0.237, $P\eta^2$ = 0.159)

List of Figures

Figure 1: Schematic representation of the experimental procedure. ($SAFT^{90}$ = 90 min soccer-simulated aerobic field test; CHO+CAFMR = Carbohydrate and caffeine mouth rinse; CHOMR = Carbohydrate mouth rinse only; CAFMR = Caffeine mouth rinse only; PLA = Placebo; FS = The Feeling Scale; FAS = The Felt Arousal Scale; RPE = The Rating of Perceived Exertion Scale).

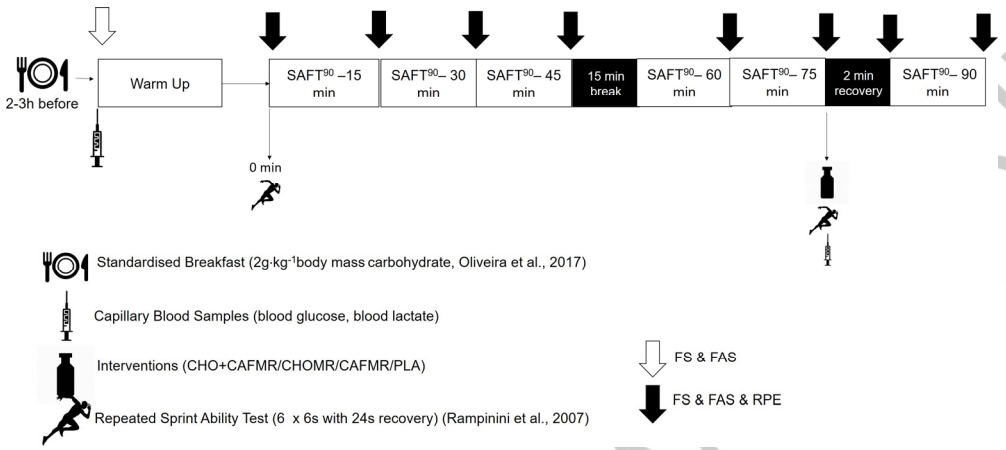

Figure 2: Mean power output (A), peak power output (B), fatigue index (C) for 1st RSAT and 2nd RSAT. (CHO+CAFMR = Caffeine and carbohydrate mouth rinse; CHOMR = Carbohydrate mouth rinse only; CAFMR = Caffeine mouth rinse only; PLA = Placebo; RSAT = Repeated sprint ability test). Individual lines patterns represent the same participant responses across each trial.

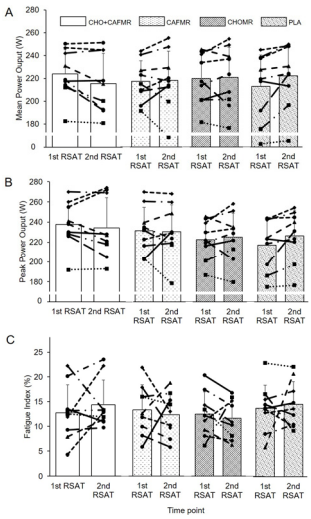

Figure 3: Blood glucose (A) and blood lactate (B) concentrations at 0 min and after 2nd RSAT. (CHO+CAFMR = Caffeine and carbohydrate mouth rinse; CHOMR = Carbohydrate mouth rinse only; CAFMR = Caffeine mouth rinse only; PLA = Placebo; RSAT = Repeated sprint ability test). Individual lines patterns represent the same participant responses across each trial.