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**CARBOHYDRATE AND CAFFEINE MOUTH RINSE AFTER 45-MIN OF AN INTERMITTENT SHUTTLE
RUN TEST DID NOT ENHANCE FOOTBALL PERFORMANCE IN COLLEGIATE PLAYERS**

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ABSTRACT

Introduction: Carbohydrate (CHO) mouth rinse improves exercise lasting ≤ 1 h, whereas caffeine (CAF) mouth rinse increases the performance of repeated bouts of short duration, high-intensity cycling. Limited research is currently available regarding the effects of a combined CHO and CAF mouth rinse on repeated sprint and skill patterns in team sports. This study examined the effect of a CHO and CAF mouth rinse on sprint running and football skill performance. **Methods:** Twenty-four male collegiate football and futsal players participated in a counterbalanced, double-blinded study where they completed 45 min of the Loughborough Intermittent Shuttle Test (LIST) on four occasions separated by at least 1 wk. Participants rinsed and expectorated 25 ml of 6.4% maltodextrin (CHO), 1.2% CAF, 6.4% maltodextrin combined with 1.2% CAF (CHO+CAF), or placebo for 10 s every 5 min after completing the LIST. Then, they performed repeated sprint ability, the Loughborough Soccer Passing Test, and Loughborough Soccer Shooting Test. **Results:** There was no significant improvement in the average ($p=0.759$) or fastest sprint times ($p=0.916$). There were no significant differences in the passing time ($p=0.756$), shooting time ($p=0.579$), and points scored per shot ($p=0.500$). There were also no significant differences in heart rate and rating of perceived exertion between the solutions. **Conclusion:** The CHO and/or CAF mouth rinse did not improve football performance including sprint running, passing, shooting, and scoring after 45 -min of intermittent exercise in collegiate male athletes.

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INTRODUCTION

Small amounts of carbohydrate (CHO) ingestion during short duration exercise (<1 h) has been shown to enhance performance¹. The benefits associated with trace CHO intake is thought to be mediated by a reaction of the central nervous system rather than a metabolic effect seen with prolonged exercise¹. To further investigate the ergogenic effect of CHO on a short duration exercise, Carter *et al.* (2004) examined the outcome of a CHO mouth rinse without ingestion during a 1-h cycling time trial and found a 2.90% improvement in performance. Increased performance was initially thought to be mediated by unidentified CHO receptors in the oral cavity, with the detection of CHO sending a signal to the brain². Subsequently, Chamber *et al.* (2009) used functional magnetic resonance imaging, imaged the subjects' brain when they rinsed their mouths with a CHO, and found activation of areas including the anterior cingulate cortex and ventral striatum. These brain regions provide a connection between the gastrointestinal tract and the emotional process, behavioural responses and cognition³. Investigations of mouth rinses and exercise performance has been expanded to other types of solutions, such as lemon juice⁴, a cooling agent⁵, bitter tasting solutions^{6,7}, and the most popular ergogenic aid, caffeine (CAF).

CAF is a popular ergogenic aid widely used both by athletes and the general population. The main purpose of CAF use is to enhance alertness, thereby increasing productivity. CAF is traditionally ingested orally; however, it can also be absorbed by adenosine receptors in the mouth, and the rate of absorption is faster by mouth than in the gastrointestinal tract⁸. A CAF mouth rinse was first investigated by Beaven *et al.* (2013), who reported a mean power improvement in high-intensity repeated cycling sprints after mouth rinsing with 1.2% CAF. An increase in the peak and mean power after mouth rinsing with a combined CAF and CHO solution was observed⁹. Subsequent investigations also found improvement in cognitive tasks following CAF mouth rinsing⁷.

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The effects of a CHO and CAF mouth rinse on intermittent exercise and team sports remains unclear. A study by Dorling and Earnest (2013) reported no differences in sprint running and repeated sprint ability (RSA) based on results of the Loughborough Intermittent Shuttle Test (LIST) after mouth rinsing with a 6.4% CHO solution¹¹, whereas Dolan (2017) reported that a CHO and CAF mouth rinse did not improve intermittent running performance. In contrast, Rollo *et al.*'s study (2015) demonstrated improved jogging speed during the self-pace block of the modified LIST following us of a CHO mouth rinse¹².

Therefore, the effects of a CHO and CAF mouth rinse on the performance of work patterns and skill activities in a team sport such as football remain unclear. More research is required to establish whether CHO and CAF mouth rinse has an effect on football-specific performance, especially in football skills such as passing or shooting. Therefore, the aim of this investigation was to examine the effect of a CHO and CAF mouth rinse on football-specific activities such as RSA, passing, and shooting in collegiate football and futsal players.

METHODS

Participants

Twenty-four male collegiate football and futsal players (football, $n=16$ and futsal, $n=8$; mean age, 20 ± 1 years; mean height, 174 ± 5 cm; mean mass, 66.5 ± 5.8 kg; mean estimated maximal oxygen uptake ($VO_{2\max}$), 47.7 ± 3.9 $ml\cdot kg^{-1}\cdot min^{-1}$) volunteered to participate in this study. All participants provided written informed consent before participating in this study, and the investigation was approved by an institutional review board (MU-CIRB 2017/013.0102). Based on Grosso and Bracken (2005) criteria¹³, participants were characterised as having low habitual caffeine consumption (0–150 mg/day).

Experimental design

Each participant was asked to an indoor sport hall on five separate occasions at the same time each day, with each occasion being separated by at least 7 d. On visit 1, participants completed the progressive multistage shuttle run test to estimate the $VO_{2\max}$ ¹⁴. On visits 2-5, they underwent the experimental trials in a randomised double-blinded, and crossover manner. An overview of this study is presented in Figure 1.

Diet and activity before the experiments

During the 24 h before each trial, participants were requested to abstain from vigorous exercise and any CAF and alcohol intake in order to maintain a normal hydration status on the day before the test by drinking at least 6-8 glasses of water and sleeping for 6-8 h. Participants were also asked to record their food and drink consumption during the 24 h before visit 1. In the morning of each trial, participants were asked to consume a meal 3 h before the testing. Participants also provided a sample of their first morning urine for the measurement of urine specific gravity (USG) (Atago, Japan) in order to ensure a normal hydration status before each trial (average USG throughout the trials 1.020 ± 0.008).

Experimental procedures

Upon arrival at the indoor sports hall (temperature $28.0\pm2.6^\circ C$, relative humidity $72.2\pm15.0\%$), the body mass of each participant was measured (Omron HBF-326[®], Omron Healthcare, Kyoto, Japan). The participants were fitted with a heart rate (HR) monitor (Polar, Electro OY, Kempele, Finland) and instructed to sit quietly for 5 min. Their water bottles were weighed in the first experimental trial, and the volume of water ingested on visit 1 was then assigned and remained the same during the following trials. On visit 1, participants performed a multistage shuttle run test, to estimate the $VO_{2\max}$ using Rambottom's table¹⁴. These data were used to determine the jogging (55% of $VO_{2\max}$) and cruising (95% of $VO_{2\max}$) speeds for the experimental trials.

On the morning of each trial, participants were asked to complete a 5-min standardised warm-up consisting of jogging, striding, and stretching exercises, and then perform 3 blocks of the LIST. Each block consisted of 15 min of 9-10 repeated cycles of walking, sprinting, jogging (at 55% $VO_{2\max}$), and running (at 95% $VO_{2\max}$) between 20-metre lines, followed by a 3-min rest period. The LIST is a set pattern of intermittent high-

intensity running and was designed to be similar to the activity pattern in a football game¹⁵ (see Nicholas *et al.*, 2000 for a detailed methodology). The running time was dictated by audio signals. HR was recorded after 5 min of rest, after the warm-up, and immediately after each block of the LIST. The rating of perceived exertion (RPE) scale¹⁶ was assessed at rest and immediately after each block of the LIST.

Following the third 15-min rest block athletes performed the mouth rinse procedure, and then completed four RSA tests, the Loughborough Soccer Passing Test (LSPT), and the Loughborough Soccer Shooting Test (LSST)¹⁷. The fastest and average sprint times were calculated from four straight-line 20-metre sprints, separated by 20 s of active recovery. Sprint time was recorded using infrared timing gates. For LSPT, participants were asked to make 16 passes against coloured boards. Passes consisted of eight long (green and blue) and eight short (yellow and red) passes. Participants were instructed to complete the trial as fast as possible while making the fewest mistakes. A penalty time was added for errors (for example, passing inaccuracy, poor ball control, or touched any cone), which was then deducted from a perfect pass. The performance time was the sum of the passing time and penalty time. In LSST, the participant was faced away from the goal with the ball, placed in the middle of the shooting zone. Participants were required to sprint and touch the top of the cone (left or right), and then they were required to return to the starting position, pass the ball to a rebound board, control the ball, and shoot at the goal while being within the shooting area. Each trial consisted of 10 shots. The point score was the average of the total cumulative points accrued from all the shots on target (see Ali *et al.*, 2007 for a detailed methodology). Performances in the LSST were the shooting time and points scored. Times were measured using a hand-held stopwatch (DX9116-A, Citizen, Tokyo, Japan).

Mouth rinse protocol

Mouth rinse solutions, 25-mL, were provided in a clear plastic cup. Participants were asked to rinse their mouth with the solution for 10 s every 5 min during the 15-min rest following the LIST protocol. The 3 rinses combined to a total of 75 ml of the solution being rinsed and expectorated during each trial (Figure 1). The solutions were as follows: 1) 6.4% maltodextrin (CHO); 2) 1.2% w/v CAF; 3) 6.4% maltodextrin with 1.2% w/v CAF (CHO+CAF) and 4) non-caloric sweetener placebo (PLA). After each rinse, participants were instructed to expectorate the solution into a pre-weighed cup. Cups were then re-weighed to determine if any of the solutions had been ingested. Participants were asked if they could guess which solution they were rinsing their mouths with. The mouth rinse solutions were taste-matched (in artificial sweetener, colouring, and flavouring) and coded by an assistant researcher to ensure double blinding. The temperature of all mouth rinse solution was neutral.

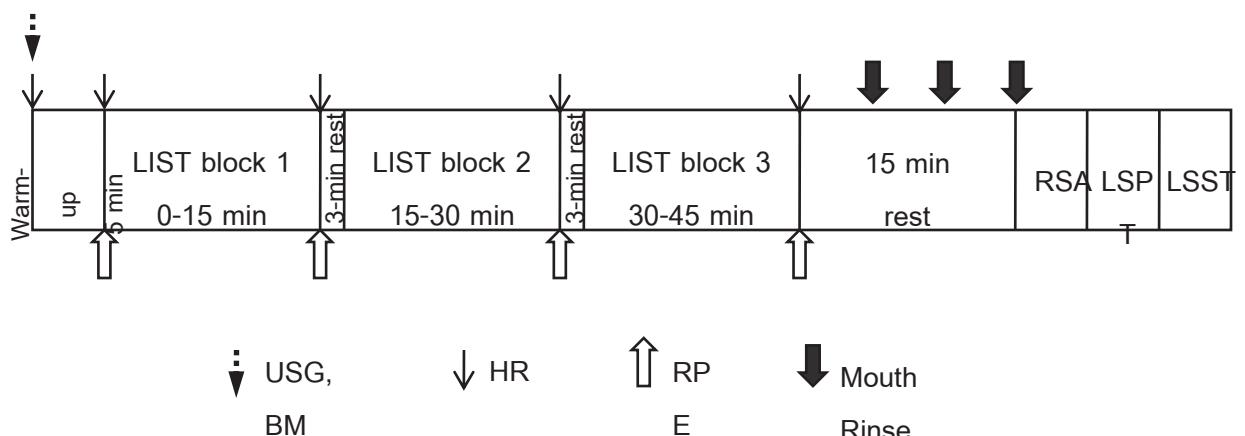


Figure 1. Schematic representation of the experimental procedures. LIST, Loughborough Intermittent Shuttle Test; LSPT, Loughborough Soccer Passing Test; LSST, Loughborough Soccer Passing Test; USG, Urine specific gravity; BM, Body mass; HR, Heart rate

Statistical Analysis

All results are presented as a mean \pm standard deviation. To determine the normality of the distribution of all data sets, the Kolmogorov-Smirnov test was used. The effects of mouth rinsing on the performance variables were analysed using one-way analysis of variance (ANOVA). A change in HR during the LIST was assessed using two-way repeated-measure ANOVA. When significant results were detected, the Bonferroni post-hoc test was used to detect between-group differences. For data sets with an abnormal distribution, the Friedman test was used to compare the effects between trials and times. Post-hoc analysis with the Wilcoxon signed-rank test was used for pairwise comparisons, resulting in a significance level set at $p<0.0125$. A paired *t*-test was used to compare changes in body mass. Statistical analyses were performed using SPSS version 18 (SPSS, IBM Corp., Armonk, NY, USA). Statistical significance was set at $p<0.05$.

RESULTS

Football performance

There were no significant differences in the fastest ($p=0.916$) and average sprint times ($p=0.759$) between all the trials (Table 1). The LSPT and LSST performance scores for all trials are shown in Table 2. Specifically, there was no significant difference in the LSPT time ($p=0.847$), penalty time ($p=0.901$), and overall performance passing time ($p=0.756$). In regards to shooting performance, there was also no significant differences in the time taken to complete each shot ($p=0.579$) and the point score per shot ($p=0.500$).

Table 1 Fastest and average sprint times in repeated sprint ability test.

Variable	CHO	CAF	CHO+CAF	PLA
Fastest time (s)	3.31±0.17	3.28±0.18	3.29±0.12	3.30±0.15
Average time (s)	3.45±0.16	3.49±0.25	3.51±0.28	3.48±0.23

Data are presented as a mean±standard deviation.

CHO, carbohydrate; CAF, caffeine; PLA, placebo

Table 2 Loughborough Soccer Passing Test and Loughborough Soccer Shooting Test performance.

Variable	CHO	CAF	CHO+CAF	PLA
LSPT				
Passing time (s)	34.25±6.60	33.65±6.15	34.80±6.94	35.21±5.89
Penalty time only (s)	0.38±4.08	-0.67±6.27	0.25±5.78	-0.13±4.41
Performance time (s)	34.66±6.81	32.98±7.52	35.05±9.32	35.08±7.07
LSST				
Shooting time (s)	8.52±0.74	8.37±0.68	8.48±0.68	8.26±0.71
Points scored	1.90±0.84	2.22±0.69	2.09±0.65	2.05±0.63

Data are presented as a mean±standard deviation.

LSPT, Loughborough Soccer Passing Test; LSST, Loughborough Soccer Shooting Test; CHO, carbohydrate; CAF, caffeine; PLA, placebo

Heart rate and rating of perceived exertion

HR increased from baseline and was significantly different between the LIST blocks ($p<0.001$) (Table 3). Specifically, significant differences were identified between blocks 1 and 2 ($p=0.017$), and between blocks 1 and 3 ($p=0.001$). However, there was no significant difference between the trials ($p=0.826$) and no interaction effect ($p=0.683$; Table 4). RPE scores increased from baseline, and there was a statistically significant difference between each block of the LIST ($p<0.001$). There were significant differences between baseline and blocks 1, 2, and 3 in all trials. However, RPE scores in each block were not significantly different between the trials (Table 4).

Table 3 Mean heart rate during the Loughborough Intermittent Shuttle Test.

Trial	Baseline	After warm up	Block 1	Block 2	Block 3
CHO	65±9	101±13 ^a	144±16 ^a	146±15 ^{a,b}	148±17 ^{a,b}
CAF	64±10	101±15 ^a	144±17 ^a	147±19 ^{a,b}	148±17 ^{a,b}
CHO+CAF	65±9	100±12 ^a	145±17 ^a	151±16 ^{a,b}	151±18 ^{a,b}
PLA	64±14	100±15 ^a	144±18 ^a	151±17 ^{a,b}	152±17 ^{a,b}

Data are presented as a mean±standard deviation.

^a Significantly different from baseline ($p<0.05$). ^b Significantly different from block 1 ($p<0.05$).

CHO, carbohydrate; CAF, caffeine; PLA, placebo; LIST, Loughborough Intermittent Shuttle Test

Table 4 Rating of perceived exertion for each trial and block.

Trial	Baseline	Block 1	Block 2	Block 3
CHO	6±1	10±2 ^a	12±2 ^{a,b}	12±3 ^{a,b}
CAF	6±1	10±2 ^a	12±2 ^{a,b}	13±3 ^{a,b,c}
CHO+CAF	7±1	11±3 ^a	12±3 ^a	13±3 ^{a,b,c}
PLA	6±0	11±2 ^a	12±3 ^{a,b}	13±3 ^{a,b,c}

Data are presented as a mean±standard deviation.

^a Significantly different from baseline ($p<0.0125$). ^b Significantly different from block 1 ($p<0.0125$). ^c

Significantly different from block 2 ($p<0.0125$).

CHO, carbohydrate; CAF, caffeine; PLA, placebo

Detection of the expectorated solution

The mean volume of expectorated solutions was 24.4±0.6 mL, 24.3±0.6 mL, 24.3±0.5 mL, and 24.0±0.7 mL for CHO, CAF, CHO+CAF, and PLA trials, respectively. There was no significant difference between the trials in expectorated solutions ($p=0.835$).

DISCUSSION

The main finding of this study was that mouth rinsing with CHO, CAF, or a combined solution (CHO+CAF) did not improve football performance in collegiate football and futsal players. Specifically, the effects of mouth rinse after completing 45 min of the LIST on sprinting, passing, and shooting performances were not different between the mouth rinse solutions.

To the best of our knowledge, this is the first investigation of the effects of a CHO and CAF mouth rinse on skill performance in football players. Previously, only one investigation examined the effects of CHO ingestion on football players' skills. Ali *et al.*'s group investigated the effect of CHO-electrolyte (CHO-E) ingestion during 90 min of the LIST in male football players¹⁸. In the CHO-E trial, the LSST performance was found to be improved,

and there was an increase in the point scored by 11%; however, the LSPT results showed a positive tendency. The authors concluded that ingestion of a CHO-E solution during prolonged intermittent exercise helped maintain football skill.

Other studies that have examined the effect of a CHO mouth rinse on intermittent exercise. In these reports, no improvement in football performance was observed which is consistent with our result, as the sprint times were not different in all the trials. Our results are similar to the findings of Dorling and Earnest; in 2013. In their study, no significant differences were found between active males who mouth rinsed with a 6.4% maltodextrin solution or water in multiple running sprints during the LIST¹¹. Another study similarly reported no significant differences in jumping and running performance efforts after mouth rinsing with either a 6% CHO or PLA (20 mL) for 10-15 s in collegiate female footballers¹⁹. However, these results conflict with the findings from Rollo *et al.*'s group who reported that a CHO mouth rinse enhanced jogging speed during the last block of the 90-min LIST¹². Rollo and colleagues suggested that the traditional LIST may not be sufficiently sensitive to detect the change in running speed in response to a mouth rinse intervention. To improve detection of changes in performance participants were allowed to self-select their speed during the last 15 min of exercise, and this change in methodology was able to identify a small performance benefit with the CHO mouth rinse.

The mechanisms behind the effects of a mouth rinse and exercise performance remain unclear. Both non-sweet (maltodextrin) and sweet (glucose) CHO mouth rinses improved motivation and activated motor control centres in the brain³. It has been proposed that the brain may notice energy input, such as CHO in the mouth and regulate energy expenditure consequently. The receptors associated with signal transduction after mouth rinses have not yet been identified. These receptors may provide a mechanistic explanation for the beneficial effects of a CHO mouth rinse on exercise performance observed in some studies^{3, 20, 21}.

Many studies have explored the effects of a mouth rinse on different exercise modalities and it appeared that mouth rinsing is beneficial during exercise and lasts for about 1 h. Results for other types of exercise remain in dispute, especially in intermittent team sports such as football. Part of the reason for different findings is the large variation in the study protocols used. Variables include mouth rinse contact time, performance tests, the calibre of participants, and whether participants were fed or fasted pre-mouth rinsing. The mouth rinse protocol used in this study was adapted from Chambers *et al.* (2009) and Beaven *et al.* (2013). Insufficient time or low frequency of mouth rinse may affect the results for performance improvement in this study. CHO mouth rinse for 10 s showed a performance improvement in comparison with exposure of 5 s²². With longer CAF mouth rinse more receptors are activated in the oral cavity²³. A dose-response relationship between the concentration of a CHO/CAF mouth rinse and football performance should be examined to ensure that a prolonged periods of mouth rinsing assists in absorption through the oral mucosa. Blood glucose concentrations and pharmacokinetics of plasma CAF concentrations should be also measured. Additional study is required to ascertain the effects of daily habitual CAF intake. It is possible that habitual caffeine intake would diminish the sensitivity to caffeine exposure in the mouth, decreasing the ergogenic effects of a CAF mouth

rinse. Additionally, in the football field setting, there is limited time for a series of mouth rinsing during the 15-min half time. Therefore, the timing of a mouth rinse in this study was designed to represent the typical opportunities for drinking in football match-play conditions (during the half-time point). Although the time allotted was practical in a football game, it may not be adequate to improve football performance.

Many studies have shown the effects of CHO mouth rinse in the fasted state were more potent in improving performance more than in a fed state^{24,25}. However, this is not always the case as seen in a study by Fares *et al.*, where performance effects in cycling time to exhaustion were observed after an overnight fast as well as 3 h after a meal²⁶. Therefore, our results are not likely to be confounded by a fed or fasted state. In our study, participants were tested in a fed state to imitate real-life situations where the intake of a meal is advised before an exercise session.

Other possible factors that have an effect on physiological and psychological variables are exercise intensity and duration. In this study, HR ranged 100-180 beats/min (bpm) (approximately 50-90% HR_{max}) with a mean HR of 150 bpm. There was a large variation in HR and the mean HR was lower than the HR typically observed during competitive match plays. In this study, HRs were recorded immediately after the end of each block, so changes in the HR might not have been detected and therefore impacted the HR data. The average RPE value at the third block of the LIST was 13±2 (i.e. somewhat hard) where exercise intensity was moderate¹⁶, consistent with the HR results. RPE values during the LIST did not change between trials, indicating that the participants performed at the same exercise intensity and that there was consistency in exercise intensity throughout the study.

Another factor that could have affected the results was lack of homogeneity in our sample population. The participants were only collegiate football and futsal players; thus the difference in fitness, playing position, and skills between football and futsal players could have affected our study's results. However, a close examination of the subjects more closely found no differences in performance between playing positions. Moreover, sprint times and point scores were similar between the subjects; whereas, passing and shooting time were likely to be lower in futsal players than in football players. Both sports involve the same physical and skill requirements but are different in the playing surface, weight of the ball, and even the type of shoes worn, all of which could have impacted the passing and shooting performances. We did not measure blood sample variables such as blood glucose or plasma CAF to determine whether there were any metabolic changes after exercise and post-mouth rinse. Finally, although solutions were closely matched for taste and colour, it was difficult to mask the bitter flavour of CAF. This made the CAF solution more identifiable than other solutions. Nevertheless, most of the participants were unable to distinguish the solutions and no side effects were reported.

CONCLUSIONS

The findings of the current study are unique, in that this is the first study to investigate the effects of a mouth rinse on football skill. We found that mouth rinsing with CHO, CAF, or a combined solution (CHO+CAF) after 45 min of an intermittent shuttle run test did not improve football performance in collegiate football and futsal players. At this time, more work is needed to fully examine the effect of CHO and CAF mouth rinsing on football performance. However, mouth rinse is still a useful strategy to consider for athletes who are sensitive to the volume of fluid in their stomach or those who suffer from gastrointestinal discomfort with food/fluid ingestion during exercise. In addition, athletes who might not have adequate time for a pre-exercise meal or require a boost at a key moment during competitions (e.g. penalty kicks, extra time) may benefit from the use of a mouth rinse.

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