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The physiological and performance effects of caffeine gum consumed during a simulated half-time by professional academy rugby union players

--Manuscript Draft--

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Corresponding Author:	Liam Patrick kilduff, PhD Swansea University Swansea, Glamorgan UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Swansea University
Corresponding Author's Secondary Institution:	
First Author:	Mark Russell
First Author Secondary Information:	
Order of Authors:	Mark Russell
	Nicholas Reynolds
	Blair Crewther
	Christian Cook
	Liam Patrick kilduff, PhD
Order of Authors Secondary Information:	
Manuscript Region of Origin:	UNITED KINGDOM
Abstract:	Despite the prevalence of caffeine as an ergogenic aid, few studies have examined the use of caffeinated gums, especially during half-time in team sports. The physiological (blood lactate, salivary hormone concentrations) and performance (repeated sprints, cognitive function) effects of consuming caffeine gum during a simulated half-time were examined. Professional academy rugby union players (n=14) completed this double-blind, randomized, counterbalanced study. Following pre-exercise measurements , players chewed a placebo (PL) gum for five min before a standardized warm-up and completing repeated sprint testing (RSSA1). Thereafter, during a 15 min simulated half-time period, players chewed either caffeine (CAF: 400 mg; 4.1 ± 0.5 mg·kg-1) or PL gum for five min before completing a second repeated sprint test (RSSA2). Blood lactate, salivary testosterone and cortisol concentrations, and indices of cognitive function (i.e., reaction time and Stroop test) were measured at baseline, pre-RSSA1, post-RSSA1, pre-RSSA2 and post-RSSA2. Sprint performance was not affected by CAF ($P=0.995$) despite slower sprint times following the first sprint of both RSSA tests (all $P<0.002$). Following half-time, salivary testosterone increased by 70% (+97±58 pg·mL-1) in CAF versus PLA ($P<0.001$) whereas salivary cortisol remained unchanged ($P=0.307$). Cognitive performance was unaffected by time and trial (all $P>0.05$). Although performance effects were absent, chewing caffeine gum increased the salivary testosterone concentrations of professional rugby union players over a simulated half-time. Practitioners may therefore choose to recommend caffeine gum between successive exercise bouts due to the increases in salivary testosterone observed; a variable associated with increased motivation and high-intensity exercise performance.

Response to Reviewers:	RE: JSCR-08-8777, entitled "The physiological and performance effects of caffeine gum consumed during a simulated half-time by professional academy rugby union players" We thank you for the opportunity to revise our manuscript and address the comments provided by the expert reviewers who have appraised our submission. A point-by-point response now follows… Reviewer #1 comments Comment 1.1: Page 2, line 35-36. The abstract states that the trial order was counterbalanced, but this does not appear to be the case. Please clarify. Response: We apologize for this omission. Action: The term counterbalanced has been added to the experimental approach to the problem and the nutritional intervention sections Comment 1.2: Consider changing "sprint attempt" to "first sprint" to avoid any confusion. Upon first reading this, I questioned whether multiple attempts were permitted for the RSSA. Response: Thank you for this recommendation Action: Amended as requested Comment 1.3: Introduction - Cohesive and well written. Response: Thank you for these kind words Action: None required Comment 1.4: Page 4, line 10-20. The addition of this statement negatively affects the flow of this introduction and does not seem necessary. Consider removing. Response: Thank you for this recommendation Action: Amended as requested Comment 1.5: Page 4, line 31. "taken provided" please remove one of these terms. Response: We apologize for this error Action: Amended as requested Comment 1.6: Page 5, lines 5-7. The abstract states that the study design was counterbalanced, but this is not stated in the methods section. Please clarify. Response: Please see response to comment 1.1 – we can clarify that the study was completed in a counterbalanced manner Action: As per comment 1.1 Comment 1.7: The experimental approach to the problem is generally confusing and should be revised. It should read as the first part of the methodology, but requires a preliminary reading of the entire methodology to make sense. Was familiarization conducted on a different day to the intervention portion of the study? A study schematic that clearly delineates the experimental design would be useful and clarify confusion in the procedure. Response: We thank R1 for this suggestion. We have revised this section Action: This section now reads: “A randomized, placebo-controlled, counterbalanced, crossover study design was used to examine the effects of caffeine gum consumed during a simulated half-time period that separated repeated sprint bouts. Following pre-exercise measurements and a standardized warm-up, players chewed a placebo (PL) gum for 5 min before completing repeated sprint testing (RSSA1). Thereafter, during a 15 min simulated half-time period, players chewed either caffeine (CAF: 400 mg; 4.1 ± 0.5 mg·kg-1) or PL gum for 5 min before completing a second repeated sprint test (RSSA2). Blood lactate, salivary testosterone and cortisol concentrations, and indices of cognitive function (i.e., reaction time and Stroop test) were measured at baseline, pre-RSSA1, post-RSSA1, pre-RSSA2 and post-RSSA2.” Comment 1.8: Page 6, line 17. What was the purpose of having participants chew PL gum for 5 minutes in between saliva/blood and cognitive performance testing prior to RSSA-1? Response: We required players to chew PL gum in order to control for the potential impact of chewing on salivary bio-markers and thus demonstrate that chewing itself had little impact upon the results observed when gums were consumed at half-time. On reflection we agree that the clarity of this could be improved in the submission and we thank R1 for highlighting this important point. Action: We have amended the methods section to include: “In order to control for the potential impact of chewing on salivary bio-markers,…” And, in the discussion, we have included: “…a finding independent from the action of chewing itself as no changes were observed when PLA gums were consumed at the start of each trial.”

	Comment 1.9: Page 6, line 44. What gum was consumed? Presumably this was randomized and counterbalanced, but this is not stated here. Response: The half-time consumption of the gum was indeed randomized and counterbalanced as per comment 1.1. As this gum consumption was the focus of the intervention, we refer the reader to the dedicated nutritional interventions section. Action: None required Comment 1.10: Page 7, line 7. More information is needed regarding the repeated sprint testing. Were participants given a "go" command? Were all participants assessed together? If seperate, what are the implications on outcome markers? Please elaborate on "individual sprint times". Was total sprint time or % decrement in sprint time assessed? Response: On reflection we agree that further clarification could be included in this section. We originally referenced one of the source publications for this protocol but in order to provide more detail we have amended the resubmission. Action: We have included: “Using a single setup and staggered start times, players performed the repeated sprint protocol in pairs in an order which remained consistent between trials. Each sprint was initiated by a “go” command following a three second count-down by a test administrator. Players were encouraged to sprint towards a cone placed two metres beyond the finish line in order to minimize inadvertent deceleration on approach to the 40 m mark.” into this paragraph. Comment 1.11: Page 7 line 22: Was blood lactate measured in singlet or duplicate? CV values? Response: Blood was measured in singlet Action: We have clarified this in the relevant section Comment 1.12: Page 7 line 36: One of my main concerns with the methodology of this study is the handling of the salivary samples. I am concerned with the 12-hour time frame before freezing saliva. What was done during this time? Were samples chilled or kept at room temperature. Was this consistent across trials and with all participants? Significant discussion is warranted on the potential effects of your handling strategy for testosterone and cortisol along with a review of potential implications for effects on the observed results. Response: We thank R1 for highlighting this issue and we feel that further clarification is required regarding the handling of our samples. Notably, we included the word “within” in the statement concerned on P7 in order to reflect the total time it may take for a sample to fully freeze ; thus while it may read as if a 12 h time-frame separated collection and the onset of freezing, we can confirm that this was not the case. Specifically, once samples were collected, they were sealed into a portable ice-cooled chilling box where they remained for the duration of the on-site data collection – a time frame of approx. 2 h. Unfortunately, due to the location of the testing venue in relation to the -80°C freezer used, it was not possible to instantaneously place samples in to the freezer upon collection. Given the nature of the testing, it would have been the earlier samples collected which would have shown the greatest time between collection and the onset of freezing; therefore a worst case scenario of approx. 4 h may have separated the initial baseline sample collection and freezer deposition. That said, we are confident that the measures put in place were consistent and indeed suitable for preservation of sample integrity – evidenced by the normative values demonstrated in baseline and pre-RSSA1 samples both between trials and in relation to previously published data. We have sought to clarify this process in our revision. Action: This sentence now reads: “All samples were stored at -80°C within 4 h of collection and cooled in a portable ice-chiller until freezer deposition.” Comment 1.13: Page 8 line 17. Your randomization methods for the gum are not clear. Were participants randomized to either PL or CAFF during the half time? Since this study was a cross-over, was the administration of the gum counterbalanced? Response: We thank R1 for highlighting that the clarity of this section could be improved. We have subsequently amended the specific paragraph mentioned. Action: We have amended the opening 2 sentances of this paragraph to read: “Players consumed a caffeinated (CAF) or a flavor-matched placebo (PLA) gum throughout the simulated half-time in a double-blind, randomized, counterbalanced and cross-over fashion. Trial randomization was carried out using a Latin-square design and gum pellets were provided to players by an independent person who was not involved in the main trial testing; thus a concealed allocation was used.” Comment 1.14: Was the look and taste of the gum identical in nature? Response: Yes we can confirm that the look, taste, texture and feel of the gums were the same as highlighted in the original submission via: “CAF and PLA chewing gums

were identical in shape, colour, texture and taste and were professionally formulated (Stay Alert, MarketRight Inc. USA)."
Action: None required
Comment 1.15: Page 8, Line 44. Mention of the individual ANOVA depending on the assessment would also be useful. For example, your stats mention that a 2-way ANOVA was used, but you have trial (CAFF vs PL), by time (pre-post) but also have 6 individual sprints. How was this analysis accomplished?
Response: We thank R1 for highlighting this issue to us. For clarification we have revised the statistical analysis section to account for such comments.
Action: The statistical analysis section now includes: "For parametric data (confirmed by normality and variance assessments), paired sample t-tests were performed for single time-point data. For parametric data expressed over multiple time-points (i.e., sprint and cognitive performance, and physiological variables), two-way repeated measures analyses of variance (within-participant factors: treatment × time) were performed (SPSS v20, Chicago, IL). Where significant interactions were observed, supplementation was deemed to have influenced responses and simple main effects were performed using LSD corrections as necessary."
Comment 1.16: Similar to the introduction, the discussion is generally well written and cohesive. However, as mentioned above, discussion on the handling of salivary samples is needed. Authors should also discuss the testosterone concentrations in the context of similar studies to justify that the observed concentration is within expected physiological range.
Response: Please see response to comment 1.12. As we have amended the methods section to clarify this issue, we feel that there are no longer any limitations associated with this point and thus would respectfully prefer to omit mention of the saliva sampling handling in the limitations section.
Action: None required.

Reviewer #2 comments
Comment 2.1: This experimental study examine the performance, physiological, and cognitive effects of caffeine gum, when consumed by professional academy rugby players during a simulated half-time period between repeated sprint exercise bouts. The article is well written, and the topic is interesting.
Response: We thank R2 for taking the time to appraise our submission and feel that the amendments made in response to your comments have improved our work.
Comment 2.2: Caffeine is a widely used ergogenic aid that benefits physical (11). There are other references better for this sentence. This reference studied multiple sprint running trial, not team sport performance. I would suggest (rugby matches): Caffeine-containing energy drink improves sprint performance during an international rugby sevens competition.Amino Acids. 2013 Jun;44(6):1511-9. doi: 10.1007/s00726-013-1473-5.
Caffeine-containing energy drink improves physical performance of elite rugby players during a simulated match. Appl Physiol Nutr Metab. 2013 Apr;38(4):368-74. doi: 10.1139/apnm-2012-0339.
Response: Thank you for these suggestions. We have ncluded one of these sources.
Action: The reference of Del Coso et al. (2013) has been added to our submission to support this point.
Comment 2.3: Peak concentrations of caffeine and/or its metabolites are generally realised within one and three hours of ingestion (REF). Please, include the reference. Maybe Magkos and Kavouras Crit Rev Food Sci Nutr. 2005;45(7-8):535-62.
Response: Thank you for this suggestion
Action: Amended as requested.
Comment 2.4: habitual caffeine intake (191 ± 138 mg·d-1), could affect this the outcomes of the study? The same for "refraining from prior caffeine use on the day of testing". Only the day of testing? Please, include these as limitations of the study.
Response: Amended as requested
Action: The following statement has been included in the final paragraph of the discussion: "Likewise, it is plausible that the effects of supplementation were mediated by habitual caffeine intake (i.e., 191 ± 138 mg·d-1) and the requirement to abstain from caffeinated products in the immediate pre-sampling period. Nevertheless, …."
Comment 2.5: The CAF and PLA chewing gum were professionally formulated to be identical in shape, colour, texture and taste. Did you test if participants identified the PLA or CAF? The particular taste of caffeine was replicated in the placebo?
Response: Thank you for highlighting this key point. We have amended the methods

	section to clarify that players were unable to distinguish between the CAF and PLA trials. Action: We have included the following statement in the methods section: “When asked at the end of the second trial, players were unable to distinguish between CAF and PLA.” Comment 2.6: Why not individualized the caffeine dose? e.g. number of chewing gum? Response: The decision to provide an absolute dose was made based on the mode of caffeine administration used (i.e., multiples of 100 mg pellets of gum). Without having to cut the gum pellets in to proportions, a process which would increase inter-individual and inter-trial variability of the amounts provided, it was not possible to individualise the caffeine dose provided. That said, the relatively tight ranges of gum provided relative to body mass highlight the homogeneity of our sample population. Action: None required Comment 2.7: The moderate caffeine dose of used in this study (400 mg; 4.1 ± 0.5 mg·kg-1) may also have contributed to the absence of performance-enhancing effects. I do not think so, lower doses (e.g. 3 mg·kg-1) have showed ergogenic effects in a widely sport situations (rugby, soccer, volleyball, RSA,...) Response: We thank R2 for highlighting this issue to us. On reflection, we have removed the term “moderate” from this statement Action: The term “moderate” removed from the sentence Comment 2.8: Consider the inclusion of individual data (e.g. non-responder?) This could improve the outcomes of the study. Response: We thank R2 for this suggestion Action: Fig 2 has been amended to include another panel focusing on the individuality of the testosterone response over the half-time period. Further housekeeping amendments have been made as a consequence of these suggested changes.

Title: The physiological and performance effects of caffeine gum consumed during a simulated half-time by professional academy rugby union players

Short title: Half-time caffeine gum ingestion

ABSTRACT

Despite the prevalence of caffeine as an ergogenic aid, few studies have examined the use of caffeinated gums, especially during half-time in team sports. The physiological (blood lactate, salivary hormone concentrations) and performance (repeated sprints, cognitive function) effects of consuming caffeine gum during a simulated half-time were examined. Professional academy rugby union players (n=14) completed this double-blind, randomized, counterbalanced study. Following pre-exercise measurements , players chewed a placebo (PL) gum for five min before a standardized warm-up and completing repeated sprint testing (RSSA1). Thereafter, during a 15 min simulated half-time period, players chewed either caffeine (CAF: 400 mg; 4.1 ± 0.5 $mg \cdot kg^{-1}$) or PL gum for five min before completing a second repeated sprint test (RSSA2). Blood lactate, salivary testosterone and cortisol concentrations, and indices of cognitive function (i.e., reaction time and Stroop test) were measured at baseline, pre-RSSA1, post-RSSA1, pre-RSSA2 and post-RSSA2. Sprint performance was not affected by CAF (P=0.995) despite slower sprint times following the first sprint of both RSSA tests (all P<0.002). Following half-time, salivary testosterone increased by 70% (+97±58 $pg \cdot mL^{-1}$) in CAF versus PLA (P<0.001) whereas salivary cortisol remained unchanged (P=0.307). Cognitive performance was unaffected by time and trial (all P>0.05). Although performance effects were absent, chewing caffeine gum increased the salivary testosterone concentrations of professional rugby union players over a simulated half-time. Practitioners may therefore choose to recommend caffeine gum between successive exercise bouts due to the increases in salivary testosterone observed; a variable associated with increased motivation and high-intensity exercise performance.

Key words: Ergogenic, football, rugby league, team sport, testosterone, cortisol

INTRODUCTION

Rugby union is a high-intensity and intermittent collision sport requiring players to repeatedly accelerate from one phase of play to another to compete for possession (9). Matches typically are played over two 40-min halves that are each separated by a 10-15 minute half-time break. As intermittent sports players have been reported in some studies to demonstrate reduced exercise intensities in the time immediately following half-time relative to the initial stages of a match (17), half-time has been proposed as an opportunity to optimize subsequent performance (23). Notably, nutritional intake is a key component of current half-time practices (30).

Caffeine is a widely used ergogenic aid that benefits physical (8, 12) and cognitive (29) indices of team sport performance. When absorbed by the lower gastro-intestinal tract, caffeine exerts its effect via a number of mechanisms including: adenosine receptor antagonism, enhanced glycolytic flux, increased sarcoplasmic reticulum calcium handling, attenuated interstitial potassium accumulation and hormonal stimulation (1, 7, 19). In the case of the latter, Beaven et al. (1) attributed the ergogenic effects of caffeine to its testosterone raising abilities. Notably, acute increases in pre-exercise testosterone concentrations have been reported to enhance high-intensity performance thereafter, including game outcomes in rugby union (11) and possibly relates to an increased motivational effect (2, 3) and/or a direct effect on the nervous system (16).

Peak concentrations of caffeine and/or its metabolites are generally realised within one and three hours of ingestion when the mechanisms of action are reliant upon

absorption via the lower gastrointestinal tract (18). In the last decade, the ergogenic effects of caffeine have also been attributed to the antagonism of receptors in the upper gastrointestinal tract facilitating a central modulation of motor unit activity, adenosine receptor stimulation (14) and augmented endocrine function (21). Notably, caffeinated chewing gums have become commercially available and have been associated with significantly faster absorption times when compared to a traditional pill-based administration modality (15).

Ryan et al. (24) observed improved cycling performance when caffeinated gum containing 300 mg of caffeine was provided five minutes before exercise. Interestingly, providing the same dose of caffeinated gum 60 and 120 min prior to the start of exercise negated the ergogenic effects observed. Similarly, Paton et al. identified that 3 $mg \cdot kg^{-1}$ of caffeine delivered by chewing gum delayed fatigue during repeated sprint cycling (21). Despite very few studies having investigated the effects of this novel method of caffeine delivery, early evidence suggests that caffeinated gum may benefit the performance of intermittent team sports players. The time-course of effects of action of caffeinated gums mean that they could plausibly be consumed during half-time in team sports; however, this has yet to be examined. The aim of this study was therefore to examine the performance, physiological and cognitive effects of caffeine gum consumed during a simulated half-time period in team sports players.

METHODS

Experimental approach to the problem

A randomized, placebo-controlled, counterbalanced, crossover study design was used to examine the effects of caffeine gum consumed during a simulated half-time period that separated repeated sprint bouts. Following pre-exercise measurements, players chewed a placebo (PL) gum for five min before completing a standardized warm-up and repeated sprint testing (RSSA1). Thereafter, during a 15 min simulated half-time period, players chewed either caffeine (CAF: 400 mg; 4.1 ± 0.5 $mg \cdot kg^{-1}$) or PL gum for five min before completing a second repeated sprint test (RSSA2). Blood lactate, salivary testosterone and cortisol concentrations, and indices of cognitive function (i.e., reaction time and Stroop test) were measured at baseline, pre-RSSA1, post-RSSA1, pre-RSSA2 and post-RSSA2. Each trial was separated by a 7-14 day period and was completed at least 48 h after any competitive match.

Subjects

Data is presented for 14 professional male academy rugby players (age: 18 ± 1 years; height: 1.83 ± 0.07 m; weight: 98.6 ± 10.9 kg) who played for a Welsh Rugby Union regional academy during the 2014/2015 season. The study required players to provide informed consent prior to participation (parental consent where aged <18 years) and conformed to the Code of Ethics of the World Medical Association (approved by the ethics advisory board of Swansea University). All players were considered healthy and injury-free at the time of the study and were three months into their competitive calendar. Players were recruited on the basis that they had been engaged in a full time professional rugby training program for at least two years

and were able to complete each of the performance assessments with correct technique.

Procedures

Following familiarization of procedures and quantification of habitual caffeine intake (191 ± 138 $mg \cdot d^{-1}$), players presented to the laboratory after having followed a standardized dietary intake (including refraining from prior caffeine use on the day of testing) as directed by a performance nutritionist. The activity in the 48 h period before main trial testing included a single training session that lasted no longer than 60 min and started at ~10:30 h. These sessions typically required a channel warm-up (including dynamic stretches and short sprints), technical drills and tactical practices to be performed and were characterized as low volume and low intensity. Players were advised to rest in the afternoons following training.

Upon arrival for the main trials, and following voiding of bladder and bowels, player's height and body mass were obtained before saliva and capillary blood was sampled and cognitive testing performed (baseline). In order to control for the potential impact of chewing on salivary bio-markers, players then chewed four pieces of placebo (PLA) chewing gum for five min followed by a 10 min rest period. Players provided a second saliva and capillary blood sample before completing further cognitive function tests (Pre-RSSA1).

A standardized ~20 min warm-up (including light running, dynamic stretching, speed preparation exercises and running drills) on an indoor synthetic running track preceded performance of the first repeated sprint test (RSSA1). Thereafter, saliva

and capillary blood was sampled again prior to further cognitive testing (Post-RSSA1). A simulated half-time then followed whereby players chewed four pieces of chewing gum for five min at the start of the break before saliva and capillary blood was sampled and cognitive function assessed (Pre-RSSA2). The time between measurements obtained at Post-RSSA1and Pre-RSSA2 was kept as close to 15 min as possible in an attempt to replicate a typical half-time that occurs during team-sport events (23). A two min re-warm up (included similar running drills as the initial warm up) preceded the second repeated sprint test (RSSA2). Thereafter, saliva and blood samples were obtained and cognitive tests performed (Post-RSSA2) before players performed a standardized cool down. To minimize circadian variation effects, measurement timing was consistent between trials.

Repeated Sprint Testing

The repeated sprint test used required 6 x 40 m (with a 180° turn at 20 m) timed sprints (Brower timing systems; Draper, Utah, USA) with 20 s active recovery between each attempt (13). Using a single setup and staggered start times, players performed the repeated sprint protocol in pairs in an order which remained consistent between trials. Each sprint was initiated by a “go” command following a three second count-down by a test administrator. Players were encouraged to sprint towards a cone placed two metres beyond the finish line in order to minimize inadvertent deceleration on approach to the 40 m mark. Repeated sprint performance was determined using individual sprint times.

Measurement of Blood Lactate and Salivary Hormones

Capillary blood samples were obtained singularly using a safety lancet (Safe-T-Pro Plus, Accu-Chek) and measured instantly using a portable lactate analyzer (Lactate Pro, Arkray, Inc., Tokyo, Japan). Saliva collection required passive drooling (~2 ml) into a sterile vial (SalivaBio, Salimetrics LLC, USA) after refraining from brushing of teeth, drinking hot fluids or eating hard foods in the two hours before sampling. All samples were stored at -80°C within four hours of collection and cooled in a portable ice-chiller until freezer deposition. Post thawing, centrifugation (Micro Centaur, MSE, London, United Kingdom; five min at 3000 revolutions·min^{-1}) preceded duplicate analysis of testosterone and cortisol concentrations (indirect enzyme-linked immunosorbent assay kits; Salimetrics Europe Ltd., Suffolk, UK). The lowest detection limits for testosterone and cortisol were 6.1 pg·mL^{-1} and 0.012 μg·dL^{-1} respectively, and inter-assay CV values were <10.0%.

Cognitive Function Testing

A simple reaction time test was completed on a laptop which measured the reaction time to visual stimuli with varying delay intervals. A total of 10 attempts were completed per test and mean reaction time was determined. The Stroop test was completed on a laptop and the percentage of correct answers to congruent and incongruent conditions were subsequently determined. The participant completed 27 responses per time point including congruent and incongruent conditions (25). Outlier detection during cognitive function analysis was classed as data that was four standard deviations away from the player's mean and was therefore removed from all statistical analysis (4).

Nutritional Interventions

Players consumed a caffeinated (CAF) or a flavor-matched placebo (PLA) gum throughout the simulated half-time in a double-blind, randomized, counterbalanced and cross-over fashion. Trial randomization was carried out using a Latin-square design and gum pellets were provided to players by an independent person who was not involved in the main trial testing; thus a concealed allocation was used. In order to control for the potential impact of chewing on salivary bio-markers, all players chewed (for five min and subsequently expectorated) four pellets of PLA gum prior to the first repeated sprint test in both trials. During half-time, four pellets of intervention gum were provided and chewed for five min before expectoration. CAF and PLA chewing gums were identical in shape, color, texture and taste and were professionally formulated (Stay Alert, MarketRight Inc. USA). Players consumed 400 mg of caffeine (i.e., four 100 mg pellets; 4.1 ± 0.5 $mg \cdot kg^{-1}$) during half-time in CAF. When asked at the end of the second trial, players were unable to distinguish between CAF and PLA.

Statistical Analysis

All data is presented as mean ± SD and an alpha level of $P \leq 0.05$ denoted significance. For parametric data (confirmed by normality and variance assessments), paired sample t-tests were performed for single time-point data. For parametric data expressed over multiple time-points (i.e., sprint and cognitive performance, and physiological variables), two-way repeated measures analyses of variance (within-participant factors: treatment × time) were performed (SPSS v20, Chicago, IL). Where significant interactions were observed, supplementation was

deemed to have influenced responses and simple main effects were performed using LSD corrections as necessary.

RESULTS

Repeated Sprint Testing

Individual sprint times are presented in Figure 1. Half-time caffeine ingestion had no effect on sprint performance (time x trial interaction: $F_{(11,143)}$=0.231, P=0.995, partial-eta^2=0.017) whereas exercise did (time effect: $F_{(5,58)}$=54.354, P<0.001, partial-eta^2=0.807). Sprint times increased after the first attempt in both RSSA tests (all P<0.002). Notably, the first sprint of RSSA2 (i.e., sprint 7) was 3% (+0.21 s) slower than the opening sprint of RSSA1 (P=0.002).

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

Physiological Responses

Exercise influenced blood lactate concentrations (time effect: $F_{(2,23)}$=286.950, P<0.001, partial-eta^2=0.957) with significant increases (P<0.001) from baseline (1.3 ± 0.6 mmol·L^{-1}) for all values collected after the post-RSSA1time-point (13.1 ± 1.6 mmol·L^{-1}). Notably mean lactate concentrations at pre-RSSA2 were still elevated above baseline (9.3 ± 3.8 mmol·L^{-1}; P<0.001) and post-RSSA2 values were 13.7 ± 1.9 mmol·L^{-1}. Half-time caffeine ingestion had no effect on blood lactate concentrations (time x trial interaction: $F_{(2,20)}$=0.181, P=0.778, partial-eta^2=0.014).

Half-time caffeine ingestion influenced salivary testosterone responses to exercise (time x trial interaction: $F_{(3,32)}$=12.070, P<0.001, partial-eta^2=0.481; Figure 2A, B) with

values at pre-RSSA2 being 70% (97 ± 58 pg·mL^{-1}) greater in CAF versus PLA (P<0.001). Individual responses to the half-time consumption of CAF are presented in Figure 2B. No further between-trial effects were observed. Likewise, salivary testosterone also increased throughout exercise (time effect: $F_{(4,52)}$=15.123, P<0.001, partial-eta^2=0.538) with values post-RSSA2 being 38% (55 pg·mL^{-1}) greater than baseline values (P<0.001). No differences were observed between baseline and pre-RSSA1 (P=0.569).

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

Exercise influenced salivary cortisol concentrations (time effect: $F_{(2,24)}$=51.864, P<0.001, partial-eta^2=0.800; Figure 2C) with significant increases from baseline occurring at the pre-RSSA2 (+81%, +0.148 μg·dL^{-1}) and post-RSSA2 (+126%, + 0.231 μg·dL^{-1}) time-points (both P<0.001). Half-time caffeine ingestion had no effect on salivary cortisol concentrations (time x trial interaction: $F_{(2,22)}$=1.226, P=0.307, partial-eta^2=0.086).

Cognitive Function Testing

Reaction time was not affected by trial (time x trial interaction: $F_{(2,31)}$=0.731, P=0.510, partial-eta^2=0.053) or time (time effect: $F_{(2,32)}$=2.940, P=0.058, partial-eta^2=0.184) with mean results being 282 ± 57 ms (Table 1). Likewise, percent correct answers on the incongruent (time x trial interaction: $F_{(4,52)}$=1.257, P=0.299, partial-eta^2=0.088; time effect: $F_{(4,52)}$=0.347, P=0.845, partial-eta^2=0.026) and congruent (time x trial interaction: $F_{(4,52)}$=0.858, P=0.495, partial-eta^2=0.062; time effect: $F_{(4,52)}$=1.109,

P=0.362, partial-eta^2=0.079) aspects of the Stroop test were not influenced over the duration of the trials being 93 ± 7% and 94 ± 8%, respectively (Table 1).

***** INSERT TABLE 1 NEAR HERE *****

DISCUSSION

The primary aim of this study was to examine the performance, physiological, and cognitive effects of caffeine gum when consumed by professional academy rugby players throughout a simulated half-time period. Our findings indicated that salivary testosterone concentrations were elevated above placebo values at the onset of a second repeated sprint bout following consumption of 4.1 ± 0.5 $mg \cdot kg^{-1}$ of caffeine in gum form. While no subsequent performance effects were observed, and no between-trial differences existed for blood lactate, salivary cortisol, or cognitive function, practitioners may wish to consider using caffeinated gum throughout half-time due to substantial increases in salivary testosterone over half-time; a variable previously associated with increased voluntary motivational effects and subsequent high-intensity exercise performance in strong (i.e., maximal squat exceeding twice body mass) individuals.

Caffeine ingestion has been reported to improve intermittent sprint performance by some (12) but not all (6, 20) authors. In this study, 400 mg of caffeine, equivalent to 4.1 ± 0.5 $mg \cdot kg^{-1}$, did not improve performance in a repeated sprint test performed shortly after (Figure 1). Although the exact reasons to explain the absence of performance improvements are difficult to ascertain, it is worth noting that the time between consumption and the onset of exercise appears to mediate the ergogenic effects of caffeinated gum (24). Notably, although sampling resolution could be improved, durations of longer than five min between consumption and the start of cycling exercise negated benefits to performance (24). As gums were chewed in the first five min of half-time, a period of ~10 min would have elapsed prior to the onset

of exercise. It is plausible that the study design used here was not optimal to capturing the window of ergogenic effect. However, this remains to be confirmed with a timing study that better explores the efficacy of caffeine gum consumption.

The findings of increased salivary testosterone concentrations in CAF support those of a previous study (1) but are the first to be reported following the half-time administration of caffeine in chewing gum form. Notably, despite similar mean testosterone concentrations (i.e., ~161 $pg \cdot mL^{-1}$) between conditions immediately before half-time (i.e., post-RSSA1), a 70% (i.e., +97 ± 58 $pg \cdot mL^{-1}$; Figure 2A) difference in values was realized in the majority of players (Figure 2B) ~15 min later after chewing CAF gum; a finding independent from the action of chewing itself as no changes were observed when PLA gums were consumed at the start of each trial. As discussed by Paton et al. (21), the magnitude and speed of such a change warrants further investigation as it appears to support the premise that testosterone can be elevated by mechanisms other than the classical hypothalamus–pituitary–gonadal axis which typically demonstrates a lag phase of ~40 min from stimulation to systemic testosterone appearance (28). Notably, albeit in rats, direct neural links have been identified between the para-ventricular nucleus of the hypothalamus and the testes (26). Our data could be interpreted to further support the idea that the rapid increase in testosterone could have been elicited via a direct neural pathway.

Free testosterone is a strong individual predictor of subsequent exercise performance in individuals with relatively high strength levels (i.e., maximum squat >2 x body mass) but a poor predictor in less strong (maximum squat <1.9 x body

mass) individuals (5). Accordingly, the apparent disconnect between the elevated testosterone concentrations observed over half-time in CAF and subsequent repeated sprint performance may be explained by the fact that although the players were professional youth rugby players engaged in full-time training and competition, compared to their senior counterparts they would be considered to be less strong. Unfortunately strength data is not available to further explore this speculation. Nevertheless, a finding of a 70% increase in testosterone concentrations over a 15 min period may have important implications for practitioners irrespective of the strength of the participant; especially, as testosterone influences subsequent exercise motivation (3) and event outcome (11).

A novel aspect of this study was the administration of the 400 mg caffeine dose via a commercially available chewing gum throughout a simulated half-time period. As reported by Paton et al. (21), the caffeine chewing gum used was not associated with any symptoms of gastrointestinal distress. Therefore, despite an absence of performance-enhancing effects in this study, chewing gum appears to be a convenient mode of administering caffeine; especially during times such as half-time whereby opportunities to ingest caffeine by traditional means (i.e., pills, drinks etc.) may be limited.

The use of the RSSA test in this study may be questioned; however, the use of such a protocol is representative of previous half-time research (22) and standardizes the physiological demands elicited between repeated trials; thus enhancing the repeatability of exercise responses (as demonstrated by pre-intervention data from RSSA1). Moreover, repeated sprint ability is associated with activity rates during

actual match-play in rugby union players (27) and the pattern of blood lactate concentrations, a surrogate marker of exercise intensity, aligns with previous authors (10). Likewise, the depressed performance at the onset of RSSA2 relative to the opening sprint of RSSA1 supports team sport literature (17) and demonstrates further that primarily passive half-time practices are not sufficient to rescue performance and/or physiological responses (i.e., blood lactate concentrations) back to the level of a comparable time-point in the first half. The dose of caffeine used in this study (400 mg; 4.1 ± 0.5 $mg \cdot kg^{-1}$) may also have contributed to the absence of performance-enhancing effects but the safety and logistical implications of chewing more than four pellets of gum at once had to be considered. Likewise, it is plausible that the effects of supplementation were mediated by habitual caffeine intake (i.e., 191 ± 138 $mg \cdot d^{-1}$) and the requirement to abstain from caffeinated products in the immediate pre-sampling period. Nevertheless, while acknowledging the impact of these limitations, applied practitioners should consider the use of caffeine gum towards the end of the half-time period; partly due to the possible ergogenic effect that a 70% increase in salivary testosterone could have thereafter.

PRACTICAL APPLICATIONS

This data adds to the developing body of literature related to both the performance and physiological responses elicited following administration of half-time interventions in team sports players. Practitioners and coaches should be cognisant of the fact that a simulated 15 min half-time was not sufficient to rescue the level of repeated sprint performance observed in the opening sprints of a prior exercise bout. Moreover, chewing caffeinated gum provides a practical and logistically feasible method of administering caffeine; especially in times when traditional methods of supplementing this ergogenic aid (e.g., pills and drinks) may not be appropriate. Although physical and cognitive performance indices were not influenced by caffeine gum, practitioners seeking enhanced testosterone concentrations should consider recommending caffeinated gum to their players. Further research is required to optimize the use of caffeinated gums (e.g., dose, timing etc.) during the half-time period.

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FIGURE LEGENDS

Figure 1: Mean ± SD 40 m sprint times throughout the caffeine (CAF; dashed line, black markers) and placebo (PLA; solid line, hollow markers) gum trials. HT represents half-time.

Figure 2: Mean ± SD salivary testosterone (panel A) and cortisol (panel C) concentrations throughout the caffeine (CAF; dashed line, black markers) and placebo (PLA; solid line, hollow markers) gum trials. Panel B represents individual half-time responses to the CAF trial (dashed line represents mean response). Shaded region represents timing of gum intake. * represents significant between-trial difference

Table 1: Mean ± SD simple reaction time (RT) and Stroop test percentages of correct answers in incongruent and congruent conditions throughout caffeine (CAF) and placebo (PLA) trials.

Title: The physiological and performance effects of caffeine gum consumed during a simulated half-time by professional academy rugby union players

Short title: Half-time caffeine gum ingestion

Authors: Russell, M.[1], Reynolds, N.A.[2], Crewther, B.T.[4],Cook, C.J.[3], Kilduff, L.[2,5]

Affiliations:

1 School of Social and Health Sciences, Leeds Trinity University, Leeds, United Kingdom
2 Applied Sports Technology Exercise and Medicine Research Centre (A-STEM), Swansea University, Swansea, United Kingdom
3 School of Sport, Health and Exercise Sciences, Bangor University, Bangor, United Kingdom
4 Institute of Sport-National Research Institute, Warsaw, Poland.
5 Welsh Institute of Performance Sciences (WIPS), Swansea University, Swansea, United Kingdom

Corresponding author: Professor Liam Kilduff

l.kilduff@swansea.ac.uk

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From: Liam Kilduff

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Dear *Journal of Strength and Conditioning Research* editorial board members,

Please find attached a revised copy of the original investigation manuscript entitled "The physiological and performance effects of caffeine gum consumed during a simulated half-time by professional academy rugby union players" that I wish to be re-submitted to your journal.

As per the original submission, I, as the corresponding author, together with all of the authors named, have read and approved the final re-submitted manuscript. This article has not been published elsewhere and it is not being considered for publication elsewhere, nor will it be submitted for publication elsewhere until a final decision has been made as to its acceptability by the *JSCR* editorial board. No external financial support has been received during any stage of preparation of this manuscript and no authors have any financial or other interest in the products or distributor of the products named in the study. This study was approved by the Swansea University ethics committee and the authors declare no previous interaction with *JSCR* regarding the submission of this manuscript.

May I take this opportunity to thank you in anticipation of your prompt course of action regarding the submission and I eagerly await your comments.

Yours sincerely,

Professor Liam Kilduff

Figure

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Table

Table 1: Mean ± SD simple reaction time (RT) and Stroop test percentages of correct answers i
conditions throughout caffeine (CAF) and placebo (PLA) trials.

Variable	**Condition**	**Timing**			
		Baseline	**Pre-RSSA1**	**Post-RSSA1**	**Pre-RSSA2**
Incongruent (% Correct)	PLA	94 ± 8	94 ± 7	94 ± 8	93 ± 8
	CAF	94 ± 6	91 ± 11	92 ± 6	94 ± 8
Congruent (% Correct)	PLA	90 ± 12	94 ± 6	96 ± 6	93 ± 9
	CAF	94 ± 6	94 ± 8	95 ± 6	97 ± 7
Simple RT (ms)	PLA	274 ± 34	274 ± 33	298 ± 60	302 ± 88
	CAF	274 ± 67	266 ± 50	274 ± 50	299 ± 59