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Caffeinated Gum Does Not Influence RPE-Regulated Cadence in Recreationally-Active College Females Regardless of Habitual Caffeine Consumption

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ABSTRACT

International Journal of Exercise Science 14(2): 1375-1387, 2021. Caffeine (CAF) has been extensively studied for its ergogenic and analgesic effects during exercise. However, the majority of these studies have been conducted in male populations. This study investigated the effects of acute CAF chewing gum on self-selected exercise intensity during a rating of perceived exertion (RPE) production trial in active females (n = 16, 21.0 ± 2.8 y). Data were also analyzed based on habitual CAF consumption level. Participants completed a $\dot{V}O_{2peak}$ trial, followed by a familiarization and two randomized, triple-blinded experimental RPE production trials on an arm ergometer [clamped resistance, blinded to self-selected cadence (CAD)] with either CAF gum (300 mg; 4.8 ± 0.7 mg/kg^{-1} body mass) or placebo (PLA), at a prescribed RPE of 4 and 7 (10 min each). Self-selected CAD did not statistically differ (p > 0.05) between CAF or PLA for an RPE4 (37.7 ± 1.6 vs. 37.6 ± 1.6 rev·min^{-1}) or RPE7 (42.9 ± 1.6 vs. 41.2 ± 1.7 rev·min^{-1}), respectively. There were no statistical differences between treatment groups for any other variables, except restlessness rating which was significantly higher (3.5 vs. 2.2; p = 0.03, d = 0.64) for the CAF group compared to PLA. Secondary analysis revealed no statistical differences for any variables between habitual consumers of low (23 ± 20 mg/day) or mod/high (195 ± 93 mg/day) CAF. Our data support previous studies examining CAF in women across different testing modalities and suggest that regardless of habitual CAF consumption, females might require higher doses of CAF to replicate subjective and physiological responses commonly observed using similar RPE production protocols in male participants. These findings support the need for additional investigations into female physiological and perceptual responses following CAF ingestion.

KEY WORDS: Sport nutrition, ergogenic aid, habituation, supplement, perception

INTRODUCTION

Caffeine (CAF) is well-established as an ergogenic aid in sports performance and has been reported to alter mechanisms such as increasing intracellular calcium release from the sarcoplasmic reticulum, enhancing lipolytic enzymes and free fatty acid mobilization, and blocking adenosine receptors [A_1 and/or A_{2A}; (12)]. Among these mechanisms, the most viable explanation for an ergogenic potential is CAF effect on the central nervous system as an analgesic, acting as an adenosine receptor antagonist. Binding of CAF to the A_1 and A_{2A}

receptors increases the release of neurotransmitters such as norepinephrine and dopamine and synergistically blunts an athlete's perception of pain and rating of perceived exertion [RPE; (36)]. In an athletic context, these mechanisms have far reaching implications as subjective ratings such as RPE are well documented as a valid and practical field measure for athletes and coaches alike (23).

The "RPE production" model is a generally accepted approach for regulating intensity (32). During exercise, individuals are prescribed an RPE and subsequently required to adjust their physical perception and cadence (CAD) to match their given RPE. Considering that CAF ingestion prior to low-to-moderate intensity exercise (~55-80% $\dot{V}O_{2peak}$) attenuates muscle pain and RPE during fixed-intensity exercise (4, 11, 16), it is reasonable to speculate that CAF would alter self-selected CAD using the RPE production model, although current data are mixed. Data from our laboratory (22) found that CAF ingestion (6 mg/kg^{-1}) significantly increased self-selected cycling workloads at RPEs of 4 (~17%) and 7 (~16%). However, Cole et al. (1996) did not demonstrate any statistical changes in mean work output incorporating a similar CAF dose, testing modality, and RPE production model [RPEs: 9, 12, and 15; (10)]. Discrepancies are common among CAF studies as both authors noted a high inter-individual variability among the participants responses to CAF. These studies also incorporated cycling as the primary method for assessing changes in RPE regulated workload. Interestingly, a meta-analysis demonstrated the ergogenic effects of CAF are more likely to be observed in testing modalities incorporating musculature of the lower body compared to the upper body (39). When examining the effects of CAF on motor unit recruitment and maximal voluntary contraction in knee extensors and elbow flexors, statistical differences are only found in the knee extensors (5, 39). The lack of an ergogenic effect is likely a result of the near-maximal (> 90%) motor unit recruitment in the arms compared to the legs (~80-90%) and therefore, the arms have a smaller range for performance improvements to manifest. While only speculation and provided the above findings, it would seem appropriate to incorporate the upper body when examining CAF in an RPE production model as a means for reducing confounding variables (i.e. improvements to motor unit recruitment, strength, neural excitability, etc.) which might affect CAD selection and performance. To our knowledge, no study has yet tested this hypothesis. Additional discrepancies have also been attributed to individual differences in CAF metabolism and genes [i.e. enzyme CYP1A2 and cytochrome P-450; (40)], CAF testing doses [~3-13 mg/kg^{-1}; (34)], habitual consumption (17), and subjective responses between genders (38). Indeed, while the list of inconsistencies among CAF research is large, most notable is the continued lack of CAF research among females.

Recently, Salinero and team (2019) reported that among CAF research and physical performance, only ~13% of the total participant pool were females (33). The limited research in females creates a gap between sports nutrition and CAF recommendations. Research has demonstrated CAF elicits varying responses in females than males (e.g. greater changes to blood pressure and negative feelings associated with CAF use), likely due to hormonal shifts through the month although specific mechanisms remain unclear (38). Current data also suggests that females might require higher doses of CAF when subjective ratings such as RPE are collected

and that a lack of CAF responsiveness is observed in females even when categorized by habitual CAF consumption (37).

Therefore, the aim of the present study was to investigate the effect of CAF chewing gum on CAD selection using recreationally active females during an arm ergometer protocol and the RPE production model. A secondary aim was to investigate whether habitual CAF consumption altered any potential ergogenic effect in females. The present team hypothesized that CAF chewing gum would increase CAD in low, but not mod/high habitual CAF consumers.

METHODS

Participants

A total of 16 recreationally active (i.e., ≥ 3 aerobic or resistance training sessions per week, greater than 45 min per session) females were recruited to participate in this study (age: 21 ± 2.8 years; height: 1.66 ± 0.06 m; body mass: 63.9 ± 11.2 kg; body fat: 25.5 ± 6.0%; and VO_{2peak}: 21.8 ± 2.9 ml·kg^{-1}·min^{-1}). A power analysis (beta = 0.80, alpha = 0.05, standard deviation = 7 rev·min^{-1}, and effect size = 5 rev·min^{-1}) showed 15 participants were needed. Preliminary data were collected on the initial visit. Participants completed a caffeine consumption questionnaire (8), physical activity readiness questionnaire, and a general health and medical questionnaire. Additionally, participants were not excluded for taking oral contraceptives and all participants verbally agreed they were naturally menstruating and had experienced a regular menstrual cycle across the last 3 months, although ovulation was not confirmed by the investigative team. Participants then provided the principle investigator with a 24-hour dietary recall.

This study conformed to the standards set by the Declaration of Helsinki, the standards of the International Journal of Exercise Science (28), and was approved by the institutional review board of the University of North Alabama (IRB #: 076). After inclusion and exclusion criteria were met, each participant was informed of the purpose of the study and the following procedures required and expected of each participant for the duration of the study. All participants then verbally and in writing provided consent to participate in this study.

Protocol

A double-blind (i.e. research participant and investigator administering treatment), counterbalanced, randomized crossover-within design was employed to examine the effects of a commercially available CAF chewing gum on CAD selection for all participants with follow-up between participant analysis for habitual versus sparse CAF consumers. The time of day for the experimental trials was standardized for each participant however, the menstrual cycle phase was not standardized due to a lack of data demonstrating statistical differences in markers of female performance across the menstrual cycle (26). An overview of the study is shown in Figure 1.

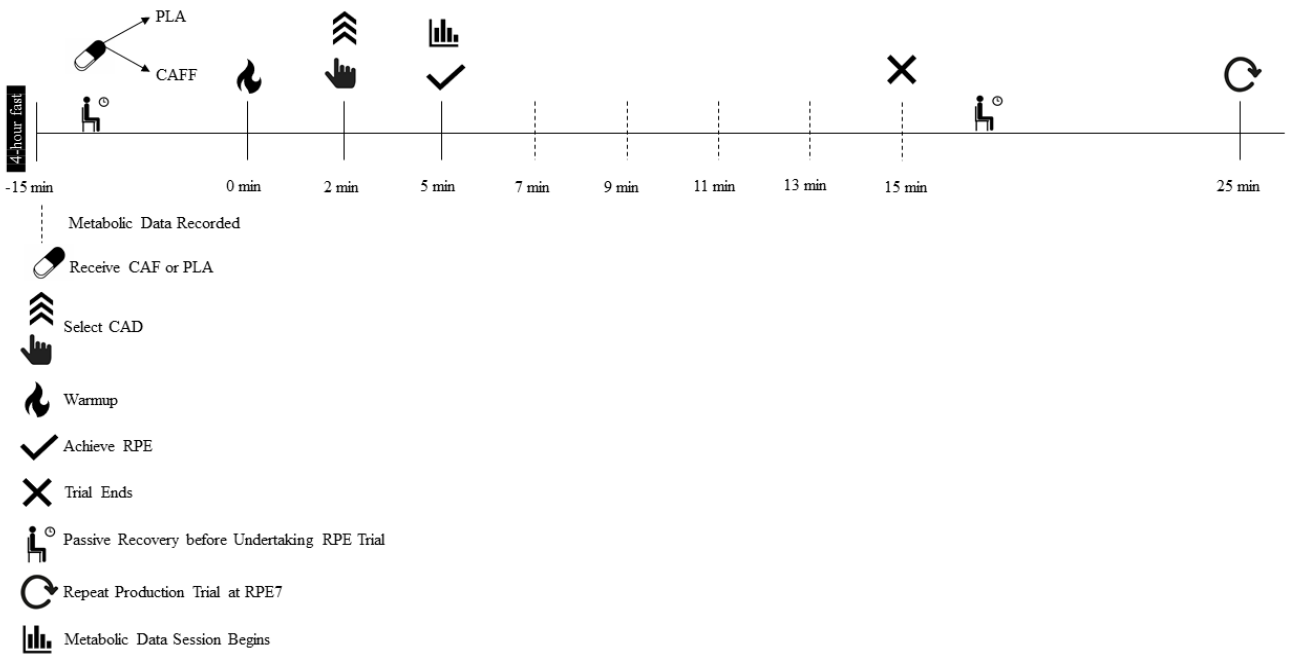

Figure 1. Schematic overview of the experimental protocol. CAF = Caffeine; PLA = Placebo; CAD = Cadence; RPE = Rating of Perceived Exertion

In brief, participants were allowed to participate in this study if they met inclusion criteria which included: (a) must meet the American College of Sports Medicine low-risk guidelines (27), (b) refraining from caffeine and alcohol consumption at least 24-hours before the experimental trials, (c) avoiding all strenuous upper-body exercise at least 48-hours before each experimental trial, and (d) be considered recreationally active as previously outlined.

Participants were asked to arrive to the laboratory between the hours of 0500 and 0900 in a non-fasted and well hydrated state (verified verbally each session). Following completion of questionnaires and obtaining informed consent, height was assessed to the nearest cm with a stadiometer (Deteco, Webb City, MO) and weight to the nearest 0.1 kg with a digital scale (Tanita Corporation, Japan). Body fat percentage was estimated using Lange skin fold calipers (Cambridge, Maryland) and a 3-site method [tricep, iliac, and thigh; (30)].

Following collection of anthropometrics, participants were then fitted with a heart rate (HR) (H10; Polar Electro Inc., Lake Success, NY, USA) monitor across their sternum and cardiorespiratory measures were collected using a Parvomedics TrueOne 2400 (Parvomedics, Sandy, UT). Correct form and posture were achieved by adjustment of seat height on the Monark arm crank ergometer (Monark Exercise 881E Rehab Trainer, Langley, WA). Handlebar position was adjusted to personal preference. Participants then completed a maximal exercise test on the arm ergometer to determine peak oxygen consumption (VO_{2peak}). Cycling began with participants required to maintain 50 ± 5 rev $\cdot min^{-1}$during the entire test. The first stage lasted for 2 min at a set workload of 15 W. After the initial stage, three consecutive stages followed which

lasted 3 min each and increased by 25 W, respectively. Following stage four, resistance then increased by 25 W every min until participants reached volitional exhaustion or could no longer maintain 50 rev·min^{-1} when provided verbal encouragement. Participants were then allowed a passive 10 min cool-down period before completing a modified familiarization trial which mimicked the experimental trials, but was shorter in duration by 5 minutes.

Following the $\dot{V}O_{2peak}$ test, participants were instructed to arrive to the lab after a minimum of 24-hours with directions to be well-rested, hydrated, and following at least a four hour fast. Additionally, the principle investigator contacted each participant 24-hours prior to their experimental trial and provide each participant their respective food logs to replicate before experimental testing the next day. Participants verbally verified these instructions prior to each visit. Participants donned appropriate instruments for the collection of metabolic data and HR, followed by seat and handlebar adjustment as described earlier. With the RPE scale in full view and information on the arm ergometer concealed, participants began the warm-up for 2 min at a self-selected pace. Following the warm-up, participants were then allowed a titration period (~3 min) and asked to gradually adjust cadence to produce an overall RPE of 4 on the OMNI Scale (6). At the completion of the titration period and following verbal confirmation from each participant they had achieved the prescribed RPE, participants then began a 10 min production trial (RPE4). The current RPE production model was adopted and modified for the Monark arm ergometer (18). Resistance for each RPE production trial was individualized and chosen based on previously published research (18). Using each participant's peak $\dot{V}O_2$, 50 rev·min^{-1}, and metabolic equations (27), a resistance was selected which approximated a 50% $\dot{V}O_2$ for RPE4 and 70% $\dot{V}O_2$ for RPE7. These intensities were clamped for each production trial and expected to fall below each participant's threshold where premature fatigue would occur and disrupt the trial. Metabolic data [i.e. $\dot{V}O_2$ and respiratory exchange ratio (RER)], HR, CAD, and RPE were recorded every 2 min (5 stages). Following completion of the 10 min production trial, participants dismounted the arm ergometer and removed metabolic collection equipment and allowed a 10 min bout of passive recovery. After the recovery period, participants then completed an identical 2 min warm-up and 3 min titration period before starting a 10 min, RPE production trial of 7 (RPE7) where identical markers were collected every 2 min. Verbal encouragement was not provided from the investigators during the RPE production trials. Upon completion of the RPE production trial, participants completed a CAF questionnaire using a ten-point Likert scale (19). For each question (fatigue, nervousness, restlessness, elevated mood, tremors, stomach distress), a zero represented not experiencing any symptom and a ten represented intense experience of the symptom. The final question provided each participant with an opportunity to choose which supplement [CAF vs. placebo (PLA)] they received during the trial.

Participants were given either 3 pieces of CAF gum (1 stick of gum = 100 mg CAF; 300 mg CAF total) or non-CAF PLA gum (Military Energy Gum; MarketRight Inc. Plano, IL, USA) approximately 15 min before performing the RPE production trials. Earlier studies examining the pharmacokinetics of CAF gum have shown 85% CAF release within 5 min of chewing and a 90% bioavailability profile (20, 34), making CAF gum a practical supplement prior to competition compared to the 60 min duration generally needed with anhydrous capsules or

powder. Both gum varieties were identical in taste, smell, and color. A researcher not involved with the RPE production trial administered the gum to each participant. All participants had previous experience with energy drinks, coffee, pre-workout supplements, and teas, but no participant had experience with CAF gum. However, following completion of the study, 9 of the 16 participants (56%) correctly guessed the CAF gum due to a bitter aftertaste left in the participants mouth once the gum had been expelled. Based on each participant's body mass, the dose of CAF used in the present study averaged 4.8 ± 0.7 mg/kg^{-1} and ranged from 3.1 to 5.8 mg/kg^{-1}. An absolute CAF dosage (300 mg) vs. a relative (mg/kg^{-1}) was chosen to best replicate athlete practices before a sporting event.

Statistical Analysis

Data are reported as mean ± *SD*. The α level was set at $p \leq 0.05$ to be considered statistically significant. Data were tested for normality using the Shapiro-Wilk's test prior to proceeding with the parametric tests as described. Sphericity was evaluated using Mauchly's test and Greenhouse-Geisser adjustments were used where appropriate. Data were first analyzed as a whole group (n = 16), regardless of CAF habitual consumption. To compare CAD (rev·min^{-1}), HR (bpm), $\dot{V}O_2$ (ml·kg^{-1}·min^{-1}), and RER between CAF and PLA, a 2 × 5 (treatment [PLA vs. CAF] × time point [5 stages]) repeated-measures analysis of variance (RMANOVA) were conducted using SPSS (Version 26; IBM, Chicago, IL). Where appropriate, follow-up paired t tests were used to compare specific time points between trials. Paired t tests were also used to compare Likert scale responses (treatment [PLA vs. CAF]). To examine whether the treatment had a true effect on CAD selection, dependent t tests (time [Trial 1 vs. Trial 2]) were carried out to identify the trial order effects, if any. Cohen's d (d: 0.2 = small, 0.5 = moderate, and 0.8 = large effect) were calculated to provide effect sizes for an interpretation of meaningful differences (9). To assess individual responses to CAF (vs. PLA), we calculated a least significant difference (LSD) for each prescribed intensity (RPE4 and RPE7). Mean values for individual participants across stages were calculated for CAF and PLA and a pooled standard deviation was determined. The LSD was calculated using the pooled standard deviation, alpha 0.05, beta 0.8, along with the number of participants (n = 16). For RPE4 the LSD was 4.4 and for RPE7 the LSD was 4.9. These values served as criterion values for each intensity. Differences less than these criterion values were 'non-responders'. Differences greater than or equal to these values were labeled as 'positive responders' (improved as result of CAF) or 'negative responders' (lower CAD as result of CAF). A secondary analysis by low (n = 8; <50 mg/day; 23 ± 20 mg/day) and mod/high (n = 8; ≥100 mg/day; 195 ± 93 mg/day) habitual CAF consumption was then performed. Percentage change in performance between PLA and CAF trials were calculated as [(Difference in CAF and PLA)/PLA*100]. These data were then incorporated into a mixed-model ANOVA to examine interaction effects, within participant effects for time points, and between participant effects for habitual (low vs. mod/high) CAF consumption. Analyses were completed for both RPE4 and RPE7. If significant interaction or main effects for treatment were found, independent t tests were used to determine which stages exhibited significant differences between low and mod/high CAF consumers.

RESULTS

An order effect was not found for our main dependent variable CAD at either RPE of 4 or 7 (Table 1). As a whole group and for an RPE4, main effects for time points were found for HR and $\dot{V}O_2$ ($p < 0.01$), but no main effect or interaction effects were found for either parameter ($p > 0.05$). Additionally, there were no significant treatment × time point interactions, main effects, or time point differences for either RER or CAD ($p > 0.05$).

At an RPE7, main effects for time points were found for HR, $\dot{V}O_2$, and RER ($p < 0.05$), but not for CAD. Regarding RER, stage 1 was higher than stage 5 (1.04 ± 0.01 vs. 0.99 ± 0.01; $p = 0.004$), however no additional differences were found between time points. There were no significant treatment × time point interactions or main effects for HR, RER, CAD, or $\dot{V}O_2$ ($p > 0.05$).

The LSD criteria for each intensity allowed for identification of individual responses to CAF. RPE4 LSD comparisons resulted in 25% ($n = 4$) of the 16 participants being positive responders and 12.5% ($n = 2$) being negative responders to CAF. For RPE7, there were 12.5% ($n = 2$) positive responders (one approaching the criteria with a delta of 4.6) and zero negative responders (though one approached the criterion with -4.2).

Regarding subjective measurements, statistical differences were found for Likert scale data (Table 2). Specifically, participants as a whole group experienced a significantly increased sense of restlessness during the CAF trial vs. PLA ($t = 2.36$, $p = 0.03$, $d = 0.64$). All other variables (fatigue, mood, nervousness, tremors, distress) demonstrated no statistical differences, although mood approached significance ($p = 0.06$) in the CAF vs. PLA group (6.3 ± 1.5 vs. 5.3 ± 1.5), respectively.

Following secondary analysis, there were no significant main effects in percentage performance changes for stage or low vs. mod/high CAF consumers for CAD, HR, RER, or $\dot{V}O_2$ during RPE4 or RPE7. There was a significant interaction for RPE7 $\dot{V}O_2$ ($p = 0.009$; Table 3). Follow up analyses revealed a higher percentage change for mod/high vs. low CAF consumers during stage 3 ($p = 0.05$) only, and approached a statistical difference ($p < 0.08$) for stages 1 and 2.

Table 1. Comparison of performance and physiological variables in college females during PLA vs. CAF trials (mean ± SD).

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
RPE - 4					
CAD					
CAF	38.3 ± 7.4	37.7 ± 7.4	37.9 ± 6.1	37.4 ± 6.0	37.3 ± 5.6
PLA	37.3 ± 6.0	38.3 ± 6.2	37.9 ± 6.5	37.2 ± 6.7	37.1 ± 6.4
Heart Rate					
CAF	112.0 ± 20.0	118.0 ± 22.0	120.0 ± 22.0	123.0 ± 24.0	124.0 ± 23.0
PLA	117.0 ± 14.0	121.0 ± 17.0	124.0 ± 18.0	125.0 ± 20.0	125.0 ± 20.0
VO_2					
CAF	10.6 ± 1.9	10.6 ± 2.3	10.9 ± 2.9	11.4 ± 2.2	11.4 ± 2.2
PLA	10.4 ± 2.1	10.9 ± 2.1	11.1 ± 2.2	10.8 ± 2.4	11.2 ± 2.4
RER					
CAF	0.98 ± 0.08	0.98 ± 0.05	0.97 ± 0.05	0.97 ± 0.05	0.96 ± 0.04
PLA	0.98 ± 0.05	0.97 ± 0.05	0.97 ± 0.04	0.97 ± 0.06	0.97 ± 0.04
RPE - 7					
CAD					
CAF	43.6 ± 7.2	43.0 ± 7.5	42.4 ± 5.9	42.4 ± 7.0	43.1 ± 8.3
PLA	42.3 ± 6.3	40.6 ± 6.9	41.6 ± 7.8	41.5 ± 7.2	40.3 ± 7.1
Heart Rate					
CAF	138.0 ± 22.0	142.0 ± 22.0	148.0 ± 19.0	151.0 ± 19.0	153.0 ± 20.0
PLA	137.0 ± 21.0	140.0 ± 23.0	142.0 ± 23.0	144.0 ± 24.0	147.0 ± 24.0
VO_2					
CAF	14.2 ± 2.5	15.0 ± 3.0	15.7 ± 3.7	15.6 ± 3.3	15.7 ± 3.7
PLA	13.9 ± 2.8	14.0 ± 3.3	14.8 ± 3.3	14.5 ± 2.6	14.9 ± 2.8
RER					
CAF	1.03 ± 0.06	1.03 ± 0.07	1.02 ± 0.06	1.01 ± 0.06	1.01 ±.0.06
PLA	1.04 ± 0.06	1.02 ± 0.05	1.00 ± 0.06	1.00 ± 0.07	0.99 ± 0.05

Table 2. Comparison of subjective responses in college females during PLA vs. CAF trials (mean ± SD).

Variables	*CAF*	*PLA*	*p*	*d*
Fatigue	6.0 ± 1.9	6.3 ± 2.1	.60	0.15
Mood	6.3 ± 1.5	5.3 ± 1.5	.06	0.67
Nervousness	1.8 ± 1.8	1.5 ± 1.4	.60	0.19
Restlessness	3.5 ± 1.9	2.2 ± 2.3	.03*	0.62
Tremors	2.4 ± 2.6	1.5 ± 2.3	.19	0.37
Distress	1.2 ± 1.9	.81 ± 1.9	.56	0.21

* = Significant difference ($p < 0.05$)

Table 3. Comparison of delta change as percentage of performance and physiological variables of low CAF consumers (n = 8) and mod/high CAF consumers (n = 8) during PLA vs. CAF trials (mean ± SD).

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
RPE - 4					
CAD					
Mod/High	4.8 ± 14.5	6.5 ± 14.0	5.3 ± 14.2	4.4 ± 14.8	4.6 ± 17.1
Low	2.8 ± 26.4	-7.1 ± 18.6	-1.7 ± 14.4	-0.2 ± 7.3	-1.0 ± 11.0
Heart Rate					
Mod/High	-3.4 ± 13.4	-1.3 ± 10.7	-0.3 ± 10.0	2.7 ± 8.8	2.1 ± 7.6
Low	-4.8 ± 16.5	-4.8 ± 15.6	-4.6 ± 14.8	-4.5 ± 13.9	-4.0 ± 11.5
VO_2					
Mod/High	7.7 ± 13.4	2.5 ± 11.4	15.6 ± 19.8	12.5 ± 16.7	6.2 ± 14.4
Low	-0.2 ± 28.2	-4.0 ± 28.8	-8.5 ± 20.9	4.5 ± 32.6	0.8 ± 23.8
RER					
Mod/High	0.3 ± 8.4	1.5 ± 5.2	1.0 ± 5	1.6 ± 5.6	1.8 ± 4.9
Low	0.9 ± 10.3	1.1 ± 8.6	0.3 ± 8.2	-0.8 ± 7.1	-2.1 ± 2.8
RPE - 7					
CAD					
Mod/High	7.1 ± 11.7	13.2 ± 15.2	8.1 ± 16.9	4.6 ± 17.1	6.1 ± 12.7
Low	-0.5 ± 4.0	0.7 ± 11.7	-0.6 ± 8.5	1.6 ± 9.3	9.4 ± 16.3
Heart Rate					
Mod/High	2.5 ± 7.3	4.3 ± 8.0	8.4 ± 11.8	8.3 ± 10.9	8.2 ± 10.6
Low	-0.1 ± 16.1	-0.4 ± 15.8	2.3 ± 16.1	3.1 ± 13.1	2.6 ± 14.4
VO_2†					
Mod/High	11.2 ± 10.4*	17.3 ± 11.6*	16.6 ± 14.2‡	7.6 ± 14.2	7.0 ± 18.5
Low	-2.9 ± 15.9	0.8 ± 21.7	-1.0 ± 18.2	8.9 ± 17.3	5.2 ± 20.2
RER					
Mod/High	1.0 ± 4.9	-0.2 ± 5.4	0.6 ± 5.4	2.1 ± 4.7	1.7 ± 5.3
Low	-1.1 ± 6.4	2.2 ± 6.1	3.9 ± 5.7	0.9 ± 7.2	2.2 ± 6.7

† = Significant interaction effect for group-treatment (p = 0.009).
‡ = Significant difference (p = 0.05) between low and mod/high CAF consumers.
* = Near significant (p < 0.08) between low and mod/high CAF consumers.

DISCUSSION

The primary aim of the present study was to examine the effects of CAF (300 mg) on CAD selection in recreationally active females during an RPE production trial (RPE4 and RPE7). Our secondary aim was to investigate whether habitual CAF consumption altered any potential ergogenic effect in females. When analyzed as an entire cohort, our data demonstrate that acute ingestion of CAF chewing gum resulted in no meaningful differences regarding HR, RER, VO_2, or CAD selection during either RPE production trial (Table 1). However, the present group did experience a moderate level (d = 0.64) of restlessness compared to PLA (Table 2). Although mod/high doses of CAF ingestion are well known to induce elevated states of anxiety, restlessness, and tremors (29), these findings may have further implications as it relates to female performance. Provided the strong relationship between an individual's precompetition subjective state (i.e. optimal arousal threshold) and their subsequent performance in competition, it may be that an elevated level of restlessness would hamper competition performance although additional research investigating this specific area in females is needed. Moreover, secondary analysis found no differences when comparing habitual CAF

consumption in females on all dependent variables. Current literature recommends an acute ingestion of ~3-9 mg/kg^{-1} of CAF prior to exercise to improve performance (12). However, ingesting a similar CAF dose within the suggested ranges demonstrated no changes to CAD regardless of habitual consumption. Our results, albeit only a single study, showed a lack of ergogenicity from acute, moderately-dosed CAF ingestion in recreationally-active females.

Results examining the effect of CAF ingestion and performance in female populations is scarce (~13%) with equivocal outcomes (2, 34). A recent review outlined these discrepancies which include the CAF dose, time of day, modality of testing, habitual consumption, and environmental conditions (29). Although as a group, our participants demonstrated no changes to CAD with CAF or PLA, this study also appears to be the first to examine female habitual CAF consumption on subjective responses and CAD selection. Following secondary analysis, our data demonstrated no statistical differences in our primary dependent variable (i.e. CAD selection) between low and mod/high habitual CAF consumption. These findings are in contrast to Evans et al. (2018) who recently demonstrated an ergogenic effect of CAF chewing gum in male athletes with low, but not high habitual CAF consumption (13). This study is particularly interesting as these data demonstrated a low-absolute CAF dose (2 gum pieces; 200 mg) mitigated decrements to repeated sprint performance (18%). Although the present study and Evans et al. (2018) implemented different testing modalities and intensities [repeated sprints (~95%) vs. RPE production trials (50 and 70%)], both studies incorporated relatively low-absolute CAF doses (300 vs. 200 mg) compared to typical relative CAF ranges (+6 mg/kg^{-1}) and both had similar ranges for habitual low CAF consumers (23 ± 20 vs. 22 ± 12 mg/day), respectively (13). However, our study incorporated a female only cohort, whereas Evans et al. (2018) incorporated an all-male cohort. The differences in genders are important to identify for future CAF investigations, since the hormonal fluctuations in females are known to impact subjective feelings following CAF ingestion and often require females to ingest higher CAF doses to replicate similar subjective states observed in males (38). These data are important as our low habitual (relative CAF dose: 4.7 ± 0.5 mg/kg^{-1}) and mod/high habitual (relative CAF dose: 4.9 ± 0.9 mg/kg^{-1}) consumers ingested relative CAF gum doses within the recommended sport nutrition guidelines (~3-6 mg/kg^{-1}) to induce an ergogenic effect prior to exercise and yet, no effect was found. It is worth speculating that higher doses of CAF might be required in female participants to elicit similar physiological and subjective responses when incorporating an RPE production trial.

Indeed, a major limitation to the current body of CAF and performance literature is that among 21 studies included in a systematic review (15), less than 10% included female participants. This is likely due to the influence of the menstrual cycle and use of oral contraceptives in CAF metabolism, making female participants a more complex cohort for CAF research. The current pooled literature does not provide evidence that a woman's menstrual cycle or use of oral contraceptives meaningfully impacts her performance. Regardless, both variables are often cited as factors which should be controlled for in CAF studies as each have been shown to affect CAF metabolism (29). Whether similar CAF doses elicit identical subjective and physiological responses between both sexes remains unclear. Steroid hormone status has been shown to influence CAF metabolism, with a delay in CAF clearance during the luteal phase (21). It has

been suggested that the rise in estradiol and progesterone during the luteal phase impairs the primary metabolic pathway of CAF, Cytochrome P-450 (21). There also appears to be a stronger link between oral contraceptive use and a delay in CAF metabolism (31) which presents a limitation to the current study. On average, 50-70% of college-age females take an oral contraceptive (14). Our team purposefully did not include/exclude potential female participants for oral contraceptive use to best represent a randomized sample of active, college-females. Oral contraceptives dramatically reduce the clearance rate of CAF and has led investigators to recommend longer durations between CAF ingestion and performance trials (29). These data are important when incorporating CAF gum which peaks in plasma concentrations between 5-15 min, compared to ~45 min with capsules (20). Whether an elevation of plasma CAF accumulation meaningfully influences its ergogenic properties is currently unknown. However, due to our incorporation of a 15 min duration between CAF gum ingestion and exercise, it is reasonable to assume that some of our participants were taking oral contraceptives which potentially delayed CAF metabolism, masked any changes to CAD, and resulted in a Type II error.

In conclusion, our results suggest that CAF gum ingestion (300 mg), 15 min prior to an RPE production trial provided no benefit to subjective responses or CAD in active females. Additionally, these results did not differ between habitual consumers of low or mod/high CAF. The present study does not refute the large body of evidence demonstrating the erogenicity of CAF in both male and female athletes. Rather, our data are intended to add to the lack of CAF research in active females and to suggest that current CAF recommendations need additional investigation before recommendations are confidently made for female athletes.

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