Evening caffeine did not improve 100 m swimming time-trials performed 60 min post-ingestion, or the next morning after sleep.

Josh W. Newbury[1], Bryan Saunders[2,3], Lewis A. Gough[1]

[1] Human Performance and Health Research Group, Centre for Life and Sport Sciences (CLaSS), Department of Sport and Exercise, Birmingham City University, Birmingham, United Kingdom

[2] Applied Physiology and Nutrition Research Group, School of Physical Education and Sport, Rheumatology Division, Faculdade de Medicina FMUSP, University of São Paulo, São Paulo 01246-903, SP, Brazil

[3] Institute of Orthopedics and Traumatology, Faculty of Medicine FMUSP, University of São Paulo, Brazil.

Running head: Caffeine had no effect on swimming performance

Corresponding author: josh.newbury@mail.bcu.ac.uk

Word count: 3900 words

Abstract

The potential ergogenic benefits of caffeine (CAF) are well known within the athletic community, often leading to its use in adolescent swimming cohorts to enhance their performance. However, it has previously been reported that CAF has sleep disturbing effects, which could be detrimental to performance over consecutive days in multi-day competitions. Moreover, the effects that evening CAF ingestion has on sleep, side-effects, and next-day performances are yet to be researched in trained adolescents. In a double-blind, randomised, crossover design, eight national-level swimmers (age: 18±1 years, height: 1.76±0.06 cm, body mass: 69.4±6.4 kg) ingested a capsule containing 3 mg·kg BM^{-1} CAF or a placebo (PLA) 60 min before an evening 100 m swimming time-trial. The next morning, sleep was analysed (Core Consensus Sleep Diary) and 100 m time-trials were repeated. Side-effects were analysed via visual analogue scales (VAS) throughout the study. No differences were found for swimming performance (p=0.911) in the evening (CAF: 59.5±7.8 s, PLA: 59.9±7.9 s, g=0.06) or morning (CAF: 59.7±7.7 s, PLA: 60.2±7.9 s, g=0.07). In addition, no group differences were found for any subjective side-effects (e.g., anxiety: p=0.468; tachycardia: p=0.859; alertness: p=0.959) or sleep parameters (e.g., sleep latency: p=0.395; total sleep time: p=0.574). These results question the use of a standardised 3 mg·kg BM^{-1} CAF ingestion strategy for 100 m swimming time-trials in trained adolescents, although objective measures may be needed to confirm that CAF does not affect sleep within this cohort.

Key words: adolescent athletes, sport nutrition, ergogenic aids, swimming, exercise.

Introduction

Competitive performances at a young age are critical to the sporting careers of swimmers (Monteiro et al., 2017), attracting adolescents to the use of nutritional ergogenic aids despite previous discouragement (Desbrow et al., 2014; Garthe & Maughan, 2018). Of these, caffeine (CAF) is the most prevalently used by swimmers aged <21 years (Shaw et al., 2016), which is unsurprising considering its well-established benefits for aerobic endurance (Southward et al., 2018), speed-based tasks (Christensen et al., 2017), and muscular strength (Grgic et al., 2018). Caffeine produces these performance enhancing effects by blocking adenosine binding sites in the central nervous system (CNS), subsequently reducing perceptions of pain and fatigue that occur from adenosine binding (Davis & Green, 2009). In turn, this action also produces secondary benefits on neurotransmitter release, such that increased levels of adrenaline, dopamine, and serotonin can act to enhance glycolytic flux and excitation-contraction coupling to offset fatigue during exercise (Fisone et al., 2004; Fredholm et al., 1999). Indeed, a combination of these factors were suggested to have improved 2 x 100 m (-1.6 s and -2.5 s, ES=0.74 and 1.41, respectively) time-trial performances when a group of trained adolescent swimmers (aged 17±2 years) ingested 250 mg (4.3±0.2 mg·kg BM^{-1}) CAF 60 min before exercise (Collomp et al., 1992). However, in the only other study involving trained adolescent swimmers (aged 14±1 years), no worthwhile improvements were found in a 400 m time-trial (-3.8 s, ES=0.08; Azizimasouleh et al., 2014). Though the latter study may have lacked ergogenic potential due to its suboptimal ingestion timing (30 min pre-exercise), there is an overall shortage of evidence to support the use of CAF by adolescent swimmers.

Caution is warranted when adolescents ingest bolus doses of CAF >3 mg·kg BM^{-1} since adverse side-effects such as tachycardia, anxiety, stomach upset, and headaches are commonplace (Temple, 2019; Sökmen et al., 2008). Furthermore, CAF is known to have a disruptive effect on sleep duration and sleep quality when ingested up to 6 h before going to bed (Drake et al., 2013; Roehrs & Roth, 2008), which is concerning from a recovery perspective as competitions require adolescents to perform at their best during the mornings (heats) in order to make it through to swimming finals. It is therefore claimed that a strategic trade-off between sleep and CAF is required when preparing for an evening swimming

final, however, to date, no research has directly investigated the impact of CAF on sleep and next-day performances in adolescents. The purpose of this research was therefore to investigate the effects that 3 mg·kg BM^{-1} CAF has on a 100 m swimming time-trial performed in the evening, and whether this ingestion impacted sleep and recovery for a follow-up 100 m time-trial the next morning.

Materials and Methods

Participants

This research took place within a high-performance, amateur swimming club whereby 12 swimmers met the research criteria (aged 16-19 years, nationally competitive). Of these, two did not volunteer to participate and two did not complete all data collection. Based on the available participants and limited time to alter the training schedule, this research therefore utilised eight participants (male=5, female=3; age: 18±1 years, body mass: 69.4±6.4 kg, height: 1.76±0.06 m) as a matter of research constraints (Lakens, 2021). This sample size was deemed appropriate for detecting a medium effect (0.50) in repeated measures ANOVA tests (power=0.86), as identifying by a priori power analysis (G*Power, v.3.1.9.4) set to significance criteria of α=0.05 and power=0.80. At the time of the study, all participants were completing eight pool (swimming volume: 42.7±0.9 km·$week^{-1}$) and four land-based training sessions·$week^{-1}$ (training time: 20.5±1.2 h·$week^{-1}$). The study was granted institutional ethical approval (Birmingham City University: Newbury/9797/R(B)/2021/Oct/HELSFAEC) in accordance with the Declaration of Helsinki, and written informed consent was provided by both the participants and their parents/guardians.

Preliminary Procedures

The study took place during the participants' normal training regime (evening: 17:30-20:30, morning: 04:45-07:00). Based on available pool allocation and the participants' education commitments, these were the closest times that replicated when heats (9:00-12:00) and finals (18:00-20:00) would occur at

swimming competitions. The participants were asked to eat as they normally would prior to competitive races, with the exception of ingesting any other caffeine or ergogenic supplements. All participants kept a photographed dietary record of all food and fluid items consumed 24 hours before the evening familiarisation, and in the time prior to the next morning's performance. The exact same dietary patterns were requested to be followed for all subsequent trials, in which the photographed records facilitated dietary replication. A trained nutritionist confirmed adherence to such practices by conducted a verbal dietary recall during baseline measures for each trial. Dietary records shown that low CAF intakes were consumed (<50 mg·day^{-1}), which was consistent with longer duration observations within this cohort (0.1±0.2 mg·kg BM·day^{-1}; Newbury et al., 2022). Despite this, all participants reported ingesting between 100-450 mg CAF (1.3-5.9 mg·kg BM·day^{-1}) 6-10 times per year at competitions. No particpants had ingested beta-alanine or creatine monohydrate in the six months preceding the study. The familiarisation trial identified that the participants were performing at 99.4% of their personal best times for this study.

Experimental Procedures

This study involved two experimental trials in a randomised, double-blind, crossover design. Upon arrival at the training venue (time: 17:30), each participant engaged in five min seated rest before giving a 5 µL sample of capillary blood from the fingertip to assess resting blood lactate ([La^{-}]) concentration (Lactate Pro 2, Arkray, Japan). The participants were then presented with visual analogue scales (VAS) to quantify baseline perceptions of nine psychological (mood, alertness, tiredness, anxiety, readiness to exercise) and physiological (tachycardia, jitters, headache, stomach upset) side-effects associated with CAF ingestion (Sökmen et al., 2008). These required a mark to be made on a 200 mm line that was labelled to suggest no symptom on the left side and severe symptom on the right side, which was adapted based on previously used VAS scales to determine gastrointestinal side-effects (Newbury et al., 2021). The distance from the left side of the VAS scale to the participant's mark was measured and divided by 20 to give an arbitrary score out of 10 (e.g., 120 mm out of 200 mm=6.0/10 arbitrary units [AU]). Participants were then given a single hydroxypropyl methylcellulose capsule (size 00, Bulk Powders, Colchester, UK) consisting of either 3 mg·kg BM^{-1} CAF (anhydrous powder; Bulk Powders,

Colchester, UK) or a cornflour placebo (PLA; ASDA, Leeds, UK). Capsules were created and randomised by a sport and exercise technician, and were administered to participants 60 min before evening time-trials.

The participants prepared for time-trials between 17:45-18:55 by first engaging in 10-20 min of a self-selected, land-based warm-up. Though individual routines varied, warm-ups typically involved skipping (3-5 min), full body mobility (5-10 min), and bodyweight strength exercises (3-5 min). Afterwards, the participants entered a 25 m pool for a 30 min standardised swimming warm-up that was prepared by the head coach. Their remaining 10-20 min was spent changing into race suits, in passive rest, and in pre-race activation work (3-5 min before time-trials). Participants were then instructed to swim a 100 m distance as quickly as possible in their preferred stroke (freestyle=4, backstroke=1, butterfly=1, breaststroke=2). After evening time-trials were complete, all participants completed 30 min cool-down consisting of low-intensity, aerobic swimming. To maximise competitiveness, time-trials were performed in two groups of four based upon their 100 m personal best times (first heat: participants 2, 5, 7, and 8; second heat: participants 1, 3, 4, and 6).

The exact same routines were followed the next morning in preparation for a follow-up 100 m swimming time-trial (time: 04:45-06:00). No CAF was supplemented in the morning to assess the possible effects that evening supplementation has on next-day performances. Each participant competed in the same races and lanes as the previous night. The exact same experimental process was repeated after two weeks, albeit with a crossover in supplement ingestion. During both trials, participants were asked to predict which supplement they had ingested (a) immediately after the evening trial, and (b) in preparation for the morning time-trial. All warm-ups, heats and lanes were the same for all time-trials.

Performance, Blood, and Perceptual Measures

Swimming was timed by two experienced swimming coaches and the mean of both times was used as the performance measure. Ratings of perceived exertion (RPE) for the whole body were collected

immediately after the warm-ups (warm-up AM, warm-up PM) and after the 100 m swimming performances (100 m AM, 100 m PM) using a 1-10 Borg scale (Borg, 1998). The previously described methods to collect [La^-] and perceptual measures (VAS scales) were conducted at seven time points: at rest on the evening (baseline PM), immediately after the evening pool warm-up (warm-up PM), immediately after the evening 100 m time-trial (100 m PM), immediately after the evening cool-down (cool-down PM), at rest in the morning (baseline AM), immediately after the morning warm-up (warm-up AM), and immediately after the morning 100 m time-trial (100 m AM).

Subjective Sleep Parameters

All participants were requested to follow their normal sleep routines, environments, and timings throughout the course of the study. Each morning upon waking, the participants completed the Core Consensus Sleep Diary (CCSD, Carney et al., 2012), which is a validated tool for assessing self-reported sleep parameters (Maich et al., 2018). The CCSD was adapted into a virtual questionnaire (Google Forms, Google, Mountain View, CA, USA) and sent to participants via instant message (WhatsApp, Menlo Park, CA, USA). The questionnaire featured six short-answer questions (1. What time did you physically get into bed? 2. What time did you intend to fall asleep? 3. What time did you actually fall asleep? 4. How long were you awake in the night? 5. What time did you wake up this morning? 6. What time did you physically get out of bed?) and one 5-point Likert scale (7. Rate your sleep quality: 1=very poor, 5=very good). Using this information, the following variables were calculated in accordance with previous research in highly trained adolescents (Ramírez et al., 2019): total sleep time, total time spent in bed, and sleep efficiency (total time in bed divided by total sleep time). Questionnaires were completed twice: (a) for the night before the evening 100 m time-trial (night one, control) and (b) for the night of the evening 100 m time-trial (night two, experimental). Answers to the questionnaires were followed up with the participants during baseline measurements to increase accuracy of reporting.

Statistical Analysis

All data was normally distributed, which was assessed via a Shapiro-Wilk test. All statistical tests were completed using SPSS (v.25, IBM, Chicago, IL, USA) with statistical significance set at $p \leq 0.05$. All data are reported as mean ± standard deviation. A two-way repeated measures ANOVA was used to determine supplement (CAF vs. PLA) x time interactions in the majority of measured variables. Sphericity (via Mauchly test) was assessed prior to conducting ANOVA analysis, and if this was violated, a Huyn-Feldt (epsilon value >0.75) or Greenhouse-Geiser (epsilon value <0.75) correction was applied. This included the analysis of the 2 x 100 m race performances (100 m PM, 100 m AM), 2 x sleep parameters (night one, night two), 4 x RPE (warm-up PM, 100 m PM, warm-up AM, 100 m AM), and 7 x physical ($[La^-]$) and perceptual (anxiety, alertness, mood, tiredness, readiness to exercise, tachycardia, jitters, headache, stomach ache) effects of CAF supplementation (baseline PM, warm-up PM, post-race PM, cool-down PM, baseline AM, warm-up AM, post-race AM). Supplement x time effect sizes were reported as partial eta squared ($P\eta^2$) and were interpreted as small (0.01-0.05), moderate (0.06-0.13), and large (>0.13) (Cohen, 1998). For statistically significant results, post-hoc comparisons were conducted using the Bonferroni correction. Between treatment effect sizes were calculated for the comparison of individual data points using the Hedge's *g* bias correction, which was deemed appropriate based on the small sample size of the study (n<20, Lakens, 2013). These effect sizes were interpreted as small (0.20-0.49), moderate (0.50-0.79), and large (>0.79) (Cohen, 1998). For 100 m performance times, the smallest worthwhile change (SWC) was calculated by multiplying the PLA standard deviation by 0.2 (Bernards, et al., 2017). Finally, sleep data were quantified for analysis by dividing min by 60 (i.e., 15 min=0.25, 10:15 PM=22.25).

Results

Participants and Supplement Predictions

Participant 9 was withdrawn from data analysis after oversleeping and missing the morning 100 m time-trial performance following CAF ingestion (reported sleep time: 03:15-10:30, sleep latency: 195 min). Participant 10 withdrew after picking up an injury between experimental trials. All eight remaining

participants predicted their supplement ingestion after racing on the evening, and before racing in the morning, with correct guesses occurring on 38% of occasions.

Swimming Performance

Over the course of the study, there were no differences identified between 100 m time-trial performances after ingesting either CAF or PLA ($F=0.1$, $p=0.911$, $P\eta^2<0.01$; Figure 1). Indeed, trivial effect sizes were found for both evening (CAF vs. PLA; 100 m PM: 59.5±7.8 vs. 59.9±7.9 s, $g=0.07$), and morning time-trial performances (CAF vs. PLA; 100 m AM: 59.7±7.7 vs. 60.2±7.9 s, $g=0.06$). Over the course of the study, only participant 1 improved performance by the SWC (-1.6 s), which was achieved during the evening trial after CAF ingestion (CAF: 59.0 s vs. PLA: 60.6 s).

Blood Lactate

Over the course of the study, no statistical differences in [La^-] production were identified between CAF and PLA groups ($F=0.7$, $p=0.649$, $P\eta^2=0.09$). However, the identification of small effect sizes suggests that there was a CAF effect within the 60 min following ingestion (CAF vs. PLA; warm-up PM: 3.1±1.6 vs. 2.5±1.6 mmol·L^{-1}, $g=0.48$; 100 m PM, 14.5±2.7 mmol·L^{-1} vs. 13.4±3.1 mmol·L^{-1}, $g=0.36$). Trivial effect sizes were calculated between CAF and PLA at all other timepoints ($g<0.20$).

Ratings of Perceived Exertion

Caffeine did not produce any statistically significant effects on RPE throughout the study ($F=0.4$, $p=0.757$, $P\eta^2=0.05$), which was evident immediately post-ingestion for both warm-up PM (CAF: 4.5±1.1 AU, PLA: 4.5±1.3 AU, $g=0.00$) and 100 m PM (CAF: 9.5±0.8 AU, PLA: 9.5±0.8 AU, $g=0.00$). Despite reporting similar ratings the next morning, small-to-moderate effect sizes suggest that there may have

been a benefit of evening CAF ingestion (CAF vs. PLA, warm-up AM: 4.0±0.8 vs. 4.3±1.0 AU, *g*=0.31; 100 m AM: 9.5±0.8 vs. 9.9±0.4 AU, *g*=0.60).

Perceptions of Physiological Side-Effects

No supplement effects were identified across the seven sampling time points for the perceived ratings of tachycardia (F=0.4, *p*=0.859, $P\eta^2$=0.06), jitteriness (F=0.4, *p*=0.888, $P\eta^2$=0.05), stomach upset (F=1.0, *p*=0.456, $P\eta^2$=0.12), or headaches (F=0.4, *p*=0.635, $P\eta^2$=0.06). Across both supplements, there were main effects for time for tachycardia (F=57.2, *p*<0.001, $P\eta^2$=0.89) and jitteriness (F=57.2, *p*<0.001, $P\eta^2$=0.89), in which their severity increased above baseline PM values in response to warm-ups and time-trials (Figure 2). Though group level stomach upset was not affected by supplement nor time, one individual (participant 2) reported an incremental increase up to very severe symptoms (>9/10) after ingesting CAF (CAF vs. PLA; 100 m PM: 5.0 vs. 1.0 AU, cool-down PM: 7.6 vs. 4.4 AU, baseline AM: 9.8 vs. 1.7 AU).

Perceptions of Psychological Side-Effects

No supplement effects occurred within the subjective psychological side-effects of anxiety (F=0.9, *p*=0.486, $P\eta^2$=0.12), tiredness (F=0.9, *p*=0.497, $P\eta^2$=0.12), alertness (F=0.2, *p*=0.959, $P\eta^2$=0.03), readiness to exercise (F=1.6, *p*=0.168, $P\eta^2$=0.19), or mood (F=1.0, *p*=0.451, $P\eta^2$=0.12). However, overall ratings of tiredness (F=7.4, *p*=<0.001, $P\eta^2$=0.51), alertness (F=6.7, *p*<0.001, $P\eta^2$=0.49), readiness to exercise (F=4.2, *p*=0.002, $P\eta^2$=0.37), and mood (F=4.7, *p*=0.001, $P\eta^2$=0.40) were all affected by time, specifically at the baseline AM time point (Figure 3).

Subjective Sleep Parameters

No statistically significant changes were identified between CAF and PLA supplements regarding all ten subjective sleep variables (Table 1). Large standard deviations were present for most variables due to the combination of the small sample size and individual sleep behaviours. However, even with such variable results, effect sizes approaching or exceeding the 'moderate' threshold (g=0.50) were observed in multiple sleep parameters during the experimental (night two) sleep, suggesting that CAF negatively impacted upon bedtime (+23 min, g=1.11), time intended to fall asleep (+19 min, g=0.61), sleep latency (+14 min, g=0.49), time spent in bed (-25 min, g=1.12), and total sleep time (-32 min, g=0.71). In comparison, only the time intended to fall asleep was reached this effect size threshold during the control (night one) sleep (CAF vs. PLA: +24 min, g=0.87).

An observation of all data showed that the morning time-trials (night two) induced an earlier wake up time (-3 h 45 min, F=49.8, p<0.001, $P\eta^2$=0.88), an earlier time to leave bed (-3 h 58 min, F=55.0, p<0.001, $P\eta^2$=0.89), a longer sleep latency (+16 min, F=9.5, p=0.018, $P\eta^2$=0.58), less overall time in bed (-4 hr 10 min, F=32.3, p=0.001, $P\eta^2$=0.82), less time spent asleep (-3 h 36 min, F=35.3, p=0.001, $P\eta^2$=0.84), and reduced sleep quality (-1.0 AU, F=16.0, p=0.005, $P\eta^2$=0.67) compared to night one. Nonetheless, the participants did not alter the time that they went to bed (+13 min, F=0.3, p=0.585, $P\eta^2$=0.05), intended to fall sleep (-15 min, F=3.8, p=0.093, $P\eta^2$=0.35), or their wake time during the night (-9 min, F=1.2, p=0.303, $P\eta^2$=0.15).

Discussion

In contrast to previous research, ingesting CAF 60 min before a 100 m swimming time-trial did not enhance performance in a group of trained male and female adolescent swimmers. Furthermore, CAF did not produce any differences in physical or psychological side-effects in comparison to the PLA supplement. However, moderate-to-large effect sizes were identified within subjective sleep parameters, suggesting that CAF may have negatively impacted sleep latency (+14 min) and total sleep time (-32 min). This finding was further supported since an excluded participant missed the morning time-trial performance after failing to sleep following evening CAF ingestion. All other participants replicated their swimming times with just 4-6 h sleep between evening and morning trials, indicating

that one poor night of sleep is not critical for short-distance swimming performance. Though 3 mg·kg BM^{-1} CAF was well tolerated in this study, the lack of ergogenic benefits questions whether the use of this supplement should be commonplace amongst competitive adolescent swimmers, or whether individualised dosing strategies are required.

The current study identified no worthwhile performance benefits (CAF vs. PLA: -0.4 s vs. SWC: -1.6 s) when CAF was ingested 60 min before 100 m time-trial swimming. This outcome contrasts previous research by Collomp et al. (1992), whereby CAF improved an initial 100 m swimming time-trial performance by -1.6 s (*g*=0.74) in regional-level male and female adolescents. The previous study also associated CAF ingestion to an increased [La^-] production (+1.0 mmol·L^{-1}) during exercise, which was also observed in this study via a small effect size (+0.9 mmol·L^{-1}, *g*=0.36). Indeed, increased [La^-] production is often reported following CAF ingestion, though whether this is a positive (increased glycolysis) or negative (impaired clearance) side-effect remains unclear (Glaister & Gissane, 2018). The current study adds to this ambiguity since 100 m time-trial performances and RPE were almost identical the following morning (11 h post-ingestion), yet with similar rates of [La^-] being produced with both CAF (13.9±3.1 mmol·L^{-1}) and PLA ingestion (14.1±2.9 mmol·L^{-1}). It is therefore speculated that methodological differences reduced the ergogenic capacity of CAF in this study, including the non-blinding of performance times, which likely gave the participants a motivational target, which based on their high training status, was able to be achieved with or without CAF ingestion. This was evidenced in the current study since the participants were all performing within 1% of their personal best 100 m swimming times, as such reducing the 'potential for improvement' following CAF ingestion (Pickering & Grgic, 2019). However, both of these issues would be present in practice, which questions whether ingesting 3 mg·kg BM^{-1} CAF 60 min before time-trial swimming is an effective competition strategy for trained adolescents.

Ingesting CAF at a dose of 3-6 mg·kg BM^{-1} 60 min prior to exercise has long been suggested to be the most ergogenic dosing strategy (Graham, 2001; Maughan et al., 2018), though this often produces equivocal performance outcomes (Guest et al., 2021). The reasons that null effects are commonly observed is typically through differing study methodologies combined with small sample sizes, with

319 factors such as diet composition (Abuhelwa et al., 2017), CAF source (Wickham & Spriet, 2018), CAF
320 habituation (Pickering & Kiely, 2019b), individual CAF metabolism rates (Pickering & Kiely, 2019b), and
321 sex hormones (Temple & Ziegler, 2011) all having a potential influence on the optimal timing and/or
322 dosage required to produce an ergogenic effect. Considering that the current study did not control for
323 genotype, menstrual cycle stage, or diet composition between participants, it is suspected that a
324 combination of these individual factors could have reduced the ergogenic response in this study,
325 especially as only a small CAF dose was ingested at a standardised time point. This is concerning for
326 adolescent swimmers since the current study conditions were designed to mimic a real-world
327 competition scenario, hence their ingestion of 3 mg·kg BM^{-1} CAF 60 min before time-trials in practice
328 could be a flawed strategy. Whether higher CAF doses (i.e., 4-6 mg·kg BM^{-1}) would produce more
329 consistent ergogenic effects is unclear (Pickering & Kiely, 2019a; Skinner et al., 2010), and not
330 recommended for all adolescents based on an increased probability of adverse side-effects occurring
331 (Sökmen et al., 2008). This therefore points towards the importance of identifying personalised CAF
332 doses, timings, and sources in practice, with future research required to compare the effectiveness of
333 these individual strategies versus a standard approach.

334

335 Caffeine did not produce any statistically significant sleep disturbances when ingested 4.5 h before
336 attempting to sleep, however, this should be interpreted cautiously based on the subjective
337 measurements, and the limited time (8.5 h) between evening and morning time-trials. For example, the
338 analysis of effect size calculations suggest that CAF negatively impacted upon total sleep time (-32 min,
339 g=0.71) and sleep latency (+14 min, g=0.49). This concurs with previous research by Drake et al.
340 (2013), who also observed similar effects after ingesting 400 mg CAF 3 h (-63 min total sleep, g=1.31;
341 +42 min sleep latency, g=0.85) and 6h (-41 min total sleep, g=0.88; +23 min sleep latency, g=0.70)
342 before a scheduled bedtime. Importantly, the authors compared subjective versus objective
343 (polysomnography) sleep parameters, identifying that participants achieved less total sleep (3 h: -14
344 min, g=0.19; 6 h: -38 min, g=0.53) and spent longer awake during the night (3 h: +20 min, g=0.49, 6 h:
345 +8 min, g=0.43) than what they perceived, whereas no differences were found after ingesting PLA
346 (objective vs. subjective: -8 min total sleep, g=0.14; +1 min wake time during the night, g=0.11). Based
347 on these findings, it is suspected that this study may have observed statistically significant CAF versus
348 PLA effects with objective measures and *ad libitum* sleeping hours. This is further supported considering

that data from participant 9 was excluded from the study after they overslept and missed the morning time-trial after evening CAF ingestion, reporting an exacerbated sleep latency (195 vs. 75 min) as the reason behind the inability to wake. Whilst these results suggest that CAF negatively impacted the sleep of adolescent swimmers, the lack of statistical significance requires further research involving a larger cohort and a comparison to objective measures.

Conducting this research in an applied setting produced limitations that could not be controlled by the study. Logistical constraints meant that only 8.5 h separated evening and morning time-trials, which restricted each participant to just 4-6 h sleep. Whilst this is common practice for highly trained swimmers (Forndran et al., 2012), the 3-4 h reduction in total sleep between the control (night one) and experimental sleeps (night two) made it difficult to examine any CAF-related effects. In addition, the frequency that time-trials could be implemented into the training programme was at the head coach's discretion, which was less flexible as each participant was in preparation for national-level competition. This meant that menstrual cycle stage could not be controlled for in the female participants, which could have influenced subjective sleep variables (Baker & Lee, 2018). Finally, participants either went back to sleep (n=2), had a nap prior to university/sixth form education (n=2), or went straight to school (n=4) after morning time-trials. Such different actions restricted the ability to compare daytime sleepiness, mood, and mental performance the day after CAF ingestion, which could all be limiting factors to performance at multi-day swimming competitions (Fullagar et al., 2015; Roehrs & Roth, 2008). Though challenging, studies of greater control are therefore necessary, possibly during competitions when education and training commitments have less impact on sleep.

In summary, highly trained adolescent swimmers received no ergogenic benefits after ingesting 3 mg·kg BM^{-1} CAF 60 min before a 100 m swimming time-trial performance. There were also no differences in the self-reported physical or psychological side-effects or RPE compared to PLA, possibly because inter-individual differences in dietary intake, genetics, and gender required more personalised CAF doses and timings to have an effect. The effects on sleep were unclear, such that no sleep parameter reached statistical significance, yet moderate effect sizes suggest that subjective measures of total sleep time and sleep latency could have been affected. This was further supported considering that one

participant's data was excluded after failing to sleep and missing a morning time-trial following CAF ingestion. To provide a better understanding of the CAF effects on sleep during swimming competitions, more controlled studies involving objective measures are required.

Acknowledgements: We would like to acknowledge Carl Grosvenor and Chris Littler for facilitating data collection.

Authorship: J.W.N. and L.A.G. conceptualised the study and formulated the methodology. J.W.N. performed the investigation and formal analyses. J.W.N. wrote the original draft manuscript. B.S. and L.A.G. reviewed and edited the manuscript. All authors approved the final version of the paper.

Conflicts of interest: B.S. (2016/50438-0 & 2021/06836-0) has been financially supported by São Paulo Research Foundation (FAPESP) and has also received a grant from Faculdade de Medicina da Universidade de São Paulo (2020.1.362.5.2). J.W.N. and L.A.G. declare no conflicts of interest.

Funding sources: All resources were funded by Birmingham City University.

References

Abuhelwa, A. Y., Williams, D. B., Upton, R. N., & Foster, D. J. (2017). Food, gastrointestinal pH, and models of oral drug absorption. *European Journal of Pharmaceutics and Biopharmaceutics*, *112*, 234-248. https://doi.org/10.1016/j.ejpb.2016.11.034

Azizimasouleh, M., Sharifi, K., Razmjoo, S., & Ahmadi, P. (2014). Effects of caffeine, L-carnitine and their combination on 400-m freestyle performance in female swimmers. *International Journal of Biosciences*, *5*(9), 1-8. https://doi.org/10.12692/ijb/5.9.1-8

Baker, F. C., & Lee, K. A. (2018). Menstrual cycle effects on sleep. *Sleep Medicine Clinics*, *13*(3), 283-294. https://doi.org/10.1016/j.jsmc.2018.04.002

Bernards, J. R., Sato, K., Haff, G. G., & Bazyler, C. D. (2017). Current research and statistical practices in sport science and a need for change. *Sports*, *5*(4), 87. https://doi.org/10.3390/sports5040087

Borg, G. (1998). *Borg's Perceived Exertion and Pain Scales*. Champaign, IL: Human Kinetics.

Carney, C. E., Buysse, D. J., Ancoli-Israel, S., Edinger, J. D., Krystal, A. D., Lichstein, K. L., et al. (2012). The consensus sleep diary: standardizing prospective sleep self-monitoring. *Sleep*, *35*(2), 287-302. https://doi.org/10.5665/sleep.1642

Christensen, P. M., Shirai, Y., Ritz, C., & Nordsborg, N. B. (2017). Caffeine and bicarbonate for speed. A meta-analysis of legal supplements potential for improving intense endurance exercise performance. *Frontiers in Physiology*, *8*, 240. https://doi.org/10.3389/fphys.2017.00240

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences.* Hillsdale, NJ: L. Erlbaum Associates.

Collomp, K., Ahmaidi, S., Chatard, J. C., Audran, M., & Prefaut, C. (1992). Benefits of caffeine ingestion on sprint performance in trained and untrained swimmers. *European Journal of Applied Physiology and Occupational Physiology*, *64*(4), 377-380. https://doi.org/10.1007/BF00636227

Davis, J. K., & Green, J. M. (2009). Caffeine and anaerobic performance. *Sports Medicine*, *39*(10), 813-832. https://doi.org/10.2165/11317770-000000000-00000

Desbrow, B., McCormack, J., Burke, L. M., Cox, G. R., Fallon, K., Hislop, M., et al. (2014). Sports Dietitians Australia position statement: sports nutrition for the adolescent athlete. *International Journal of Sport Nutrition and Exercise Metabolism*, *24*(5), 570-584. https://doi.org/10.1123/ijsnem.2014-0031

Drake, C., Roehrs, T., Shambroom, J., & Roth, T. (2013). Caffeine effects on sleep taken 0, 3, or 6 hours before going to bed. *Journal of Clinical Sleep Medicine*, *9*(11), 1195-1200. https://doi.org/10.5664/jcsm.3170

Fisone, G., Borgkvist, A., & Usiello, A. (2004). Caffeine as a psychomotor stimulant: mechanism of action. *Cellular and Molecular Life Sciences*, *61*(7), 857-872. https://doi.org/10.1007/s00018-003-3269-3

Forndran, A., Lastella, M., Roach, G. D., Halson, S., & Sargent, C. (2012). Training schedules in elite swimmers: no time to rest. In: X. Zhou & C. Sargent (Eds.), *Sleep of Different Populations*, Adelaide: Australasian Chronobiology Society, pp.6-10.

Fredholm, B. B., Bättig, K., Holmén, J., Nehlig, A., & Zvartau, E. E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews*, *51*(1), 83-133.

Fullagar, H. H., Skorski, S., Duffield, R., Hammes, D., Coutts, A. J., & Meyer, T. (2015). Sleep and athletic performance: the effects of sleep loss on exercise performance, and physiological and cognitive responses to exercise. *Sports Medicine*, *45*(2), 161-186. https://doi.org/10.1007/s40279-014-0260-0

Garthe, I., & Maughan, R. J. (2018). Athletes and supplements: prevalence and perspectives. *International Journal of Sport Nutrition and Exercise Metabolism*, *28*(2), 126-138. https://doi.org/10.1123/ijsnem.2017-0429

Glaister, M., & Gissane, C. (2018). Caffeine and physiological responses to submaximal exercise: a meta-analysis. *International Journal of Sports Physiology and Performance*, *13*(4), 402-411. https://doi.org/10.1123/ijspp.2017-0312

Goods, P. S., Landers, G., & Fulton, S. (2017). Caffeine ingestion improves repeated freestyle sprints in elite male swimmers. *Journal of Sports Science and Medicine*, *16*(1), 93-98.

Graham, T. E. (2001). Caffeine and exercise. *Sports Medicine*, *31*(11), 785-807.

Grgic, J., Pickering, C., Del Coso, J., Schoenfeld, B. J., & Mikulic, P. (2021). CYP1A2 genotype and acute ergogenic effects of caffeine intake on exercise performance: A systematic review. *European Journal of Nutrition*, *60*(3), 1181-1195. https://doi.org/10.1007/s00394-020-02427-6

Grgic, J., Trexler, E. T., Lazinica, B., & Pedisic, Z. (2018). Effects of caffeine intake on muscle strength and power: a systematic review and meta-analysis. *Journal of the International Society of Sports Nutrition*, *15*(1), 1-10. https://doi.org/10.1186/s12970-018-0216-0

Guest, N. S., VanDusseldorp, T. A., Nelson, M. T., Grgic, J., Schoenfeld, B. J., Jenkins, N. D., et al. (2021). International Society of Sports Nutrition position stand: caffeine and exercise performance. *Journal of the International Society of Sports Nutrition*, *18*(1), 1-37. https://doi.org/10.1186/s12970-020-00383-4

Kamimori, G. H., Joubert, A., Otterstetter, R., Santaromana, M., & Eddington, N. D. (1999). The effect of the menstrual cycle on the pharmacokinetics of caffeine in normal, healthy eumenorrheic females. *European Journal of Clinical Pharmacology*, *55*(6), 445-449. https://doi.org/10.1007/s002280050654

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, 863. https://doi.org/10.3389/fpsyg.2013.00863

Lakens, D (2021). Sample size justification. *PsyArXiv*. https://doi.org/10.31234/osf.io/9d3yf

Lara, B., Gutiérrez-Hellín, J., García-Bataller, A., Rodríguez-Fernández, P., Romero-Moraleda, B., & Del Coso, J. (2020). Ergogenic effects of caffeine on peak aerobic cycling power during the menstrual cycle. *European Journal of Nutrition*, *59*(6), 2525-2534. https://doi.org/10.1007/s00394-019-02100-7

Lara, B., Ruiz-Vicente, D., Areces, F., Abián-Vicén, J., Salinero, J. J., Gonzalez-Millán, C., ... & Del Coso, J. (2015). Acute consumption of a caffeinated energy drink enhances aspects of performance in sprint swimmers. *British Journal of Nutrition*, *114*(6), 908-914. https://doi.org/10.1017/S0007114515002573

Maich, K. H., Lachowski, A. M., & Carney, C. E. (2018). Psychometric properties of the consensus sleep diary in those with insomnia disorder. *Behavioral Sleep Medicine*, *16*(2), 117-134. https://doi.org/10.1080/15402002.2016.1173556

Maughan, R. J., Burke, L. M., Dvorak, J., Larson-Meyer, D. E., Peeling, P., Phillips, S. M., et al. (2018). IOC consensus statement: dietary supplements and the high-performance athlete. *International Journal of Sport Nutrition and Exercise Metabolism*, *28*(2), 104-125. https://doi.org/10.1123/ihsnem.2018-0200

Mielgo-Ayuso, J., Marques-Jiménez, D., Refoyo, I., Del Coso, J., León-Guereño, P., & Calleja-González, J. (2019). Effect of caffeine supplementation on sports performance based on differences between sexes: a systematic review. *Nutrients*, *11*(10), 2313. https://doi.org/10.3390/nu11102313

Monteiro, D., Marinho, D., Moutão, J., Vitorino, A., Antunes, R., & Cid, L. (2018). Reasons for dropout in swimmers, differences between gender and age and intentions to return to competition. *The Journal of Sports Medicine and Physical Fitness*, *58*, 180-192. https://doi.org/10.23736/S0022-4707.17.06867-0

Newbury, J. W., Cole, M., Kelly, A. L., Chessor, R. J., Sparks, S. A., McNaughton, L. R., et al. (2021). The time to peak blood bicarbonate (HCO_3^-), pH, and the strong ion difference (SID) following sodium bicarbonate ($NaHCO_3$) ingestion in highly trained adolescent swimmers. *PLoS One*, *16*(7), e0248456. https://doi.org/10.1371/journal.pone.0248456

Newbury, J.W., Foo, W.L., Cole, M., Kelly, A.L., Chessor, R.J., Sparks, S.A., et al. (2022). Nutritional intakes of highly trained adolescent swimmers before, during, and after a national lockdown in the COVID-19 pandemic. *PLoS One*, *17*(4), e0266238. https://doi.org/10.1371/journal.pone.0266238

527 Pickering, C., & Kiely, J. (2018). Are the current guidelines on caffeine use in sport optimal for everyone?
528 Inter-individual variation in caffeine ergogenicity, and a move towards personalised sports
529 nutrition. *Sports Medicine*, *48*(1), 7-16. https://doi.org/10.1007/s40279-017-0776-1

530

531 Pickering, C., & Grgic, J. (2019). Caffeine and exercise: what next? *Sports Medicine*, *49*(7), 1007-1030.
532 https://doi.org/10.1007/s40279-019-01101-0

533

534 Pickering, C., & Kiely, J. (2019a). Are low doses of caffeine as ergogenic as higher doses? A critical
535 review highlighting the need for comparison with current best practice in caffeine
536 research. *Nutrition*, *67-68*, 110535. https://doi.org/10.1016/j.nut.2019.06.016.

537

538 Pickering, C., & Kiely, J. (2019b). What should we do about habitual caffeine use in athletes? *Sports*
539 *Medicine*, *49*(6), 833-842. https://doi.org/10.1007/s40279-018-0980-7

540

541 Pruscino, C. L., Ross, M. L., Gregory, J. R., Savage, B., & Flanagan, T. R. (2008). Effects of sodium
542 bicarbonate, caffeine, and their combination on repeated 200-m freestyle performance. *International*
543 *Journal of Sport Nutrition and Exercise Metabolism*, *18*(2), 116-130.
544 https://doi.org//10.1123/ijsnem.18.2.166

545

546 Ramírez, C. A., Till, K., Beasley, G., Giuliano, P., Leduc, C., Dalton-Barron, N., et al. (2020). Sleep
547 patterns of elite youth team-sport athletes prior to and during international competition. *Science and*
548 *Medicine in Football*, *4*(1), 15-21. https://doi.org/10.1080/24733938.2019.1662081

549

550 Roehrs, T., & Roth, T. (2008). Caffeine: sleep and daytime sleepiness. *Sleep Medicine Reviews*, *12*(2),
551 153-162. https://doi.org/10.1016/j.smrv.2007.07.004

552

553 Shaw, G., Slater, G., & Burke, L. M. (2016). Supplement use of elite Australian swimmers. *International*
554 *Journal of Sport Nutrition and Exercise Metabolism*, *26*(3), 249-258.
555 https://doi.org/10.1123/ijsnem.2015-0182

556

557 Skinner, T. L., Jenkins, D. G., Coombes, J. S., Taaffe, D. R., & Leveritt, M. D. (2010). Dose response
558 of caffeine on 2000-m rowing performance. *Medicine and Science in Sports and Exercise*, *42*(3), 571-
559 576. https://doi.org/10.1249/mss.0b013e3181b6668b

560

561 Sökmen, B., Armstrong, L. E., Kraemer, W. J., Casa, D. J., Dias, J. C., Judelson, D. A., et al. (2008).
562 Caffeine use in sports: considerations for the athlete. *The Journal of Strength and Conditioning*
563 *Research*, *22*(3), 978-986. https://doi.org/10.1519/JSC.0b013e3181660cec

564

565 Southward, K., Rutherfurd-Markwick, K. J., & Ali, A. (2018). The effect of acute caffeine ingestion on
566 endurance performance: a systematic review and meta–analysis. *Sports Medicine*, *48*(8), 1913-1928.
567 https://doi.org/10.1007/s40279-018-0939-8

568

569 Spriet, L. L. (2014). Exercise and sport performance with low doses of caffeine. *Sports Medicine*, *44*(2),
570 175-184. https://doi.org/10.1007/s40279-014-0257-8

571

572 Temple, J. L. (2019). Trends, safety, and recommendations for caffeine use in children and
573 adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry*, *58*(1), 36-45.
574 https://doi.org/10.1016/j.jaac.2018.06.030

575

576 Temple, J. L., & Ziegler, A. M. (2011). Gender differences in subjective and physiological responses to
577 caffeine and the role of steroid hormones. *Journal of Caffeine Research*, *1*(1), 41-48.
578 https://doi.org/10.1089/jcr.2011.0005

579

Wickham, K. A., & Spriet, L. L. (2018). Administration of caffeine in alternate forms. *Sports Medicine*, *48*(1), 79-91. https://doi.org/10.1007/s40279-017-0848-2

583 **Table 1. Sleep parameters for the night before (night one) and night of (night two) evening**
584 **supplement ingestion and time-trial performance.**

585

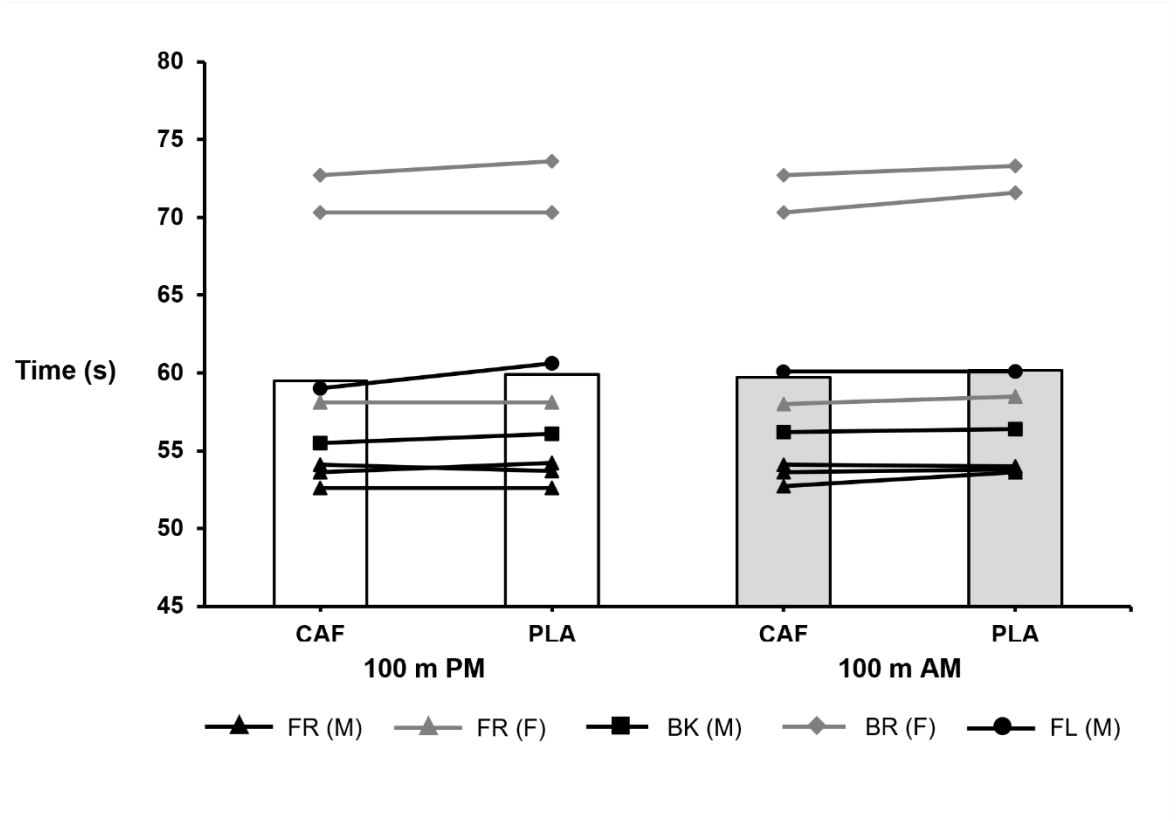
Sleep Parameter	Night One		Night Two		Interaction
	CAF	PLA	CAF	PLA	
Bedtime (h:min)	21.58±1:39	21:55±0:25	22:20±0:12	21:58±0:24	F=0.5, *p*=0.483, $P\eta^2$=0.07
Time intended to sleep (h:min)	22:55±0:30	22:30±0:23	22:36±0:20	22:17±0:32	F=0.9, *p*=0.376, $P\eta^2$=0.11
Sleep latency (min)	19±26	21±19	43±29	29±24	F=0.8, *p*=0.395, $P\eta^2$=0.11
Wake time during the night (min)	20±41	5±11	2±4	6±8	F=1.7, *p*=0.240, $P\eta^2$=0.19
Wake up time (h:min)	08:17±1:22	07:35±2:19	04:10±0:08	04:13±0:10	F=0.7, *p*=0.426, $P\eta^2$=0.09
Out of bed time (h:min)	08:41±1.29	07:47±2:25	04:14±0:07	04:17±0:08	F=1.0, *p*=0.356, $P\eta^2$=0.12
Total time spent in bed (h:min)	10:43±2:02	9:52±2:37	5:55±0:17	6:19±0:24	F=1.8, *p*=0.218, $P\eta^2$=0.21
Total sleep time (h:min)	8:44±1:20	8:39±2:10	04:49±0:34	5:20±0:49	F=0.3, *p*=0.574, $P\eta^2$=0.05
Sleep efficiency (%)	83.3±14.6	81.4±8.2	88.1±6.5	84.4±10.3	F=0.1, *p*=0.781, $P\eta^2$=0.01
Sleep quality (n/5)	4.1±0.4	4.2±0.6	3.3±0.9	3.0±0.5	F=0.4, *p*=0.563, $P\eta^2$=0.05

586

Figure 1: Individual 100 m swimming performance times following CAF and PLA ingestion in the evening (100 m PM) and in the following morning (100 m AM). FR=freestyle, BK=backstroke, BR=breaststroke, FL=butterfly, M=male swimmers, F=female swimmers.

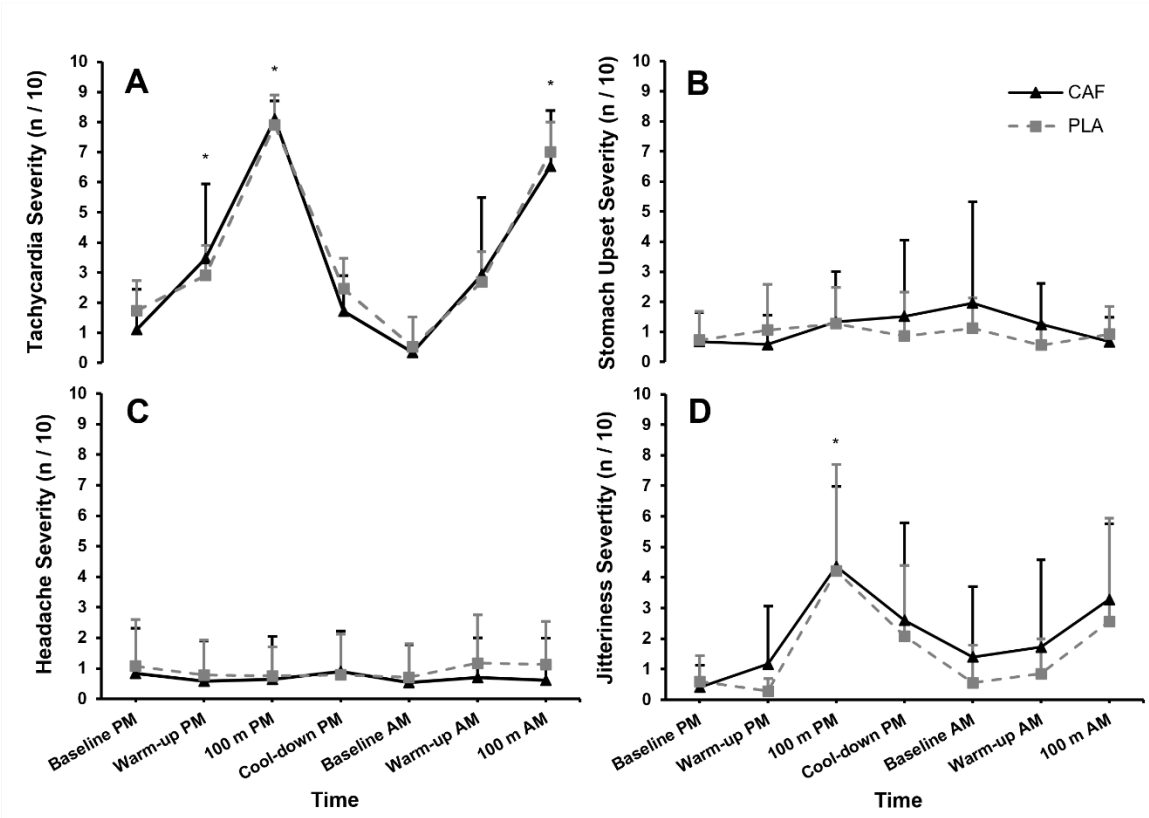
Figure 2: Subjective ratings of A) tachycardia, B) stomach upset, C) headache, and D) jitteriness following the ingestion of CAF and PLA. * denotes main effects for time compared to baseline PM ($p \leq 0.05$).

Figure 3: Subjective ratings of A) anxiety, B) tiredness, C) alertness, D) readiness to exercise, and E) mood rating following the ingestion of CAF and PLA. * denotes main effects for time compared to baseline AM ($p \leq 0.05$).


Time (s)
80
75
70
65
60
55
50
45
CAF
PLA
CAF
PLA
100 m PM
100 m AM
FR (M)
FR (F)
BK (M)
BR (F)
FL (M)

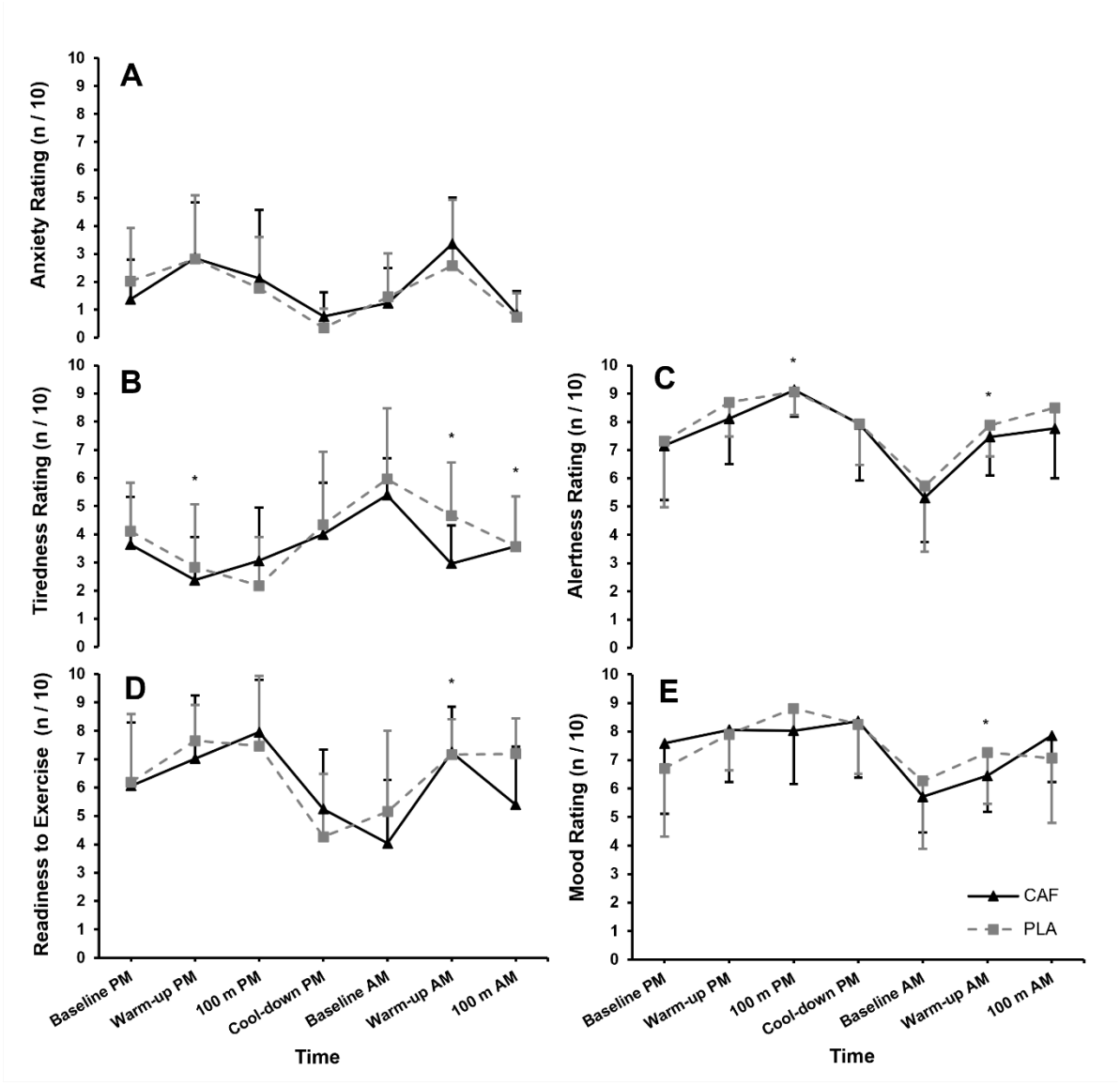
598

599


A
B
C
D
Tachycardia Severity (n / 10)
Stomach Upset Severity (n / 10)
Headache Severity (n / 10)
Jitteriness Severtity (n / 10)
CAF
PLA
Baseline PM
Warm-up PM
100 m PM
Cool-down PM
Baseline AM
Warm-up AM
100 m AM
Time

600

601


A
Anxiety Rating (n / 10)
B
Tiredness Rating (n / 10)
C
Alertness Rating (n / 10)
D
Readiness to Exercise (n / 10)
E
Mood Rating (n / 10)
CAF
PLA
Baseline PM
Warm-up PM
100 m PM
Cool-down PM
Baseline AM
Warm-up AM
100 m AM
Time

602