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Title: Timing of caffeine ingestion does not improve 3-point shooting accuracy in college basketball players.

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Running Head: Caffeine ingestion timing and basketball shooting

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Abstract

This study investigated the effects of the timing of caffeine (3 mg/kg body mass) ingestion on 3-point shooting accuracy and other performance parameters during a basketball exercise simulation test (BEST). Eighteen college basketball players (mean ± SD: age = 24.4 ± 1.5 years, height = 181.7 ± 9.5 cm, body mass = 80.9 ± 13.2 kg) underwent one familiarization trial and three main conditions in a randomized order: (i) placebo (maltodextrin) and placebo (PP), (ii) caffeine and placebo (CP) and (iii) placebo and caffeine (PC). Participants ingested either the placebo or caffeine pill 75 and 15 minutes before performing four quarters of the BEST and a 3-point shooting protocol. During each quarter, participants completed 16 rounds of the BEST and ten 3-point shots. Vertical jump height, 6 m sprint timing, BEST completion timing, 3-point shooting accuracy, heart rate (HR), rate of perceived exertion (RPE), blood glucose (BGlu), blood lactate (BLa) and psychological measures pertaining to performance were measured. The BEST completion timing differed among conditions (PP = 26.4 ± 2.0 s, CP = 25.8 ± 2.0 s, PC = 25.9 ± 2.1 s; $p = 0.031$) but not 3-point shooting accuracy (PP = 12.33 ± 4.10; CP = 12.61 ± 2.81; PC = 11.67 ± 3.77; $p = 0.648$), vertical jump height or sprint times. Manipulating ingestion timing of caffeine did not improve 3-point shooting accuracy, vertical jump height or 6 m sprint timings but caffeine can improve performance times during simulated basketball exercise irrespective of ingestion timing.

Keywords: caffeine, basketball shooting, vertical jump height, sprint timing, basketball exercise simulation test

Introduction

Caffeine can improve performance in many sports (Spriet, 2014) primarily via its action as an adenosine competitor at central and peripheral receptors reducing the dampening effects of adenosine on arousal (Davis et al., 2003) and boosting muscle firing rates (Kalmar, 2005). Basketball athletes perform repeated high-intensity movements over four quarters whilst simultaneously undertaking sport-specific skills (Abian-Vicen et al., 2014). Caffeine may be useful for these athletes by not only promoting physical performance but by attenuating fatigue-related decrements in sport-specific skills - such as shooting - as observed in other sports (Foskett et al., 2009).

Nine studies have examined caffeine's effects on components of both physical performance (e.g. aerobic capacity, agility tests, jump height, sprint times and grip strength) and sport specific basketball skills (dribbling, free throws and 3-point shooting) (Abian-Vicen et al., 2014; Filip-Stachnik et al., 2022; Puente et al., 2017; Raya-González et al., 2021; Scanlan et al., 2019; Stojanović et al., 2019; Stojanović et al., 2021; Tan et al., 2020; Tucker et al., 2013). All studies administered 3 or 6 mg/kg of caffeine in capsule or drink form 60 minutes pre-exercise, with one exception where chewing gum with ~2.3 mg/kg of caffeine was provided 15 minutes beforehand (Filip-Stachnik et al., 2022). Caffeine is often administered 60 minutes pre-exercise so estimated post-absorption peak plasma concentrations correspond with the start of exercise (Chia et al., 2017). Whether this is optimal can be challenged as caffeine may be most beneficial in sports where fatigue accumulates (Guest et al., 2021) and an intake closer to a point of greater fatigue may be more beneficial (Shen et al., 2019).

Four studies have examined the effect of caffeine on free-throw and/or 3-point shooting (Abian-Vicen et al., 2014; Filip-Stachnik et al., 2022; Puente et al., 2017; Tan et al., 2020) with only one noting improvement in free-throws alone (Puente et al., 2017). Notational analyses suggest that 3-point shooting accuracy can be vital in determining basketball match winners (Garciá et al., 2013). However, those studies investigating the use of caffeine on 3-point shooting accuracy have important limitations. One examined shooting during 20-minutes of simulated basketball game play but there were unequal numbers of shots and too few shots (< 3 shots/condition) taken on the caffeine and placebo conditions to provide a robust conclusion (Puente et al., 2017). The other two studies did not include any exercise protocol prior to shooting (Abian-Vicen et al., 2014; Filip-Stachnik et al., 2022) - for ecological validity, some game-like demand should be introduced beforehand as accuracy is negatively affected by fatigue (Mulazimoglu et al., 2017).

This study examined the effects of caffeine (3 mg/kg of body mass, BM) ingestion timing on 3-point shooting accuracy in college basketball players during simulated basketball play, with other performance parameters - vertical jump height, sprint time and basketball simulated exercise performance – as secondary outcomes. We hypothesized that caffeine administration immediately pre-exercise would improve all outcomes to a greater extent than administration 60 minutes pre-exercise, because a later peak in circulating caffeine would mitigate fatigue-related decrements in performance.

Methodology

Participants

This study was conducted in accordance with the Declaration of Helsinki and approved by Nanyang Technological University Institutional Review Board (IRB-2020-009-043-02). All participants provided written informed consent.

A priori sample size calculation suggested that 18 participants were required to elicit a statistical power of 0.95 with moderate effect size (f) of 0.40 and alpha (*p*-value) of 0.05 (Puente et al., 2017). Twenty male college basketball players were screened and 18 (mean ± SD: age = 24.4 ± 1.5 years; height = 181.7 ± 9.5cm; body mass = 80.9 ± 13.2 kg) included (Figure 1). Eligibility criteria were: (1) aged 21-30 years, (2) having competed in at least one college-level basketball competition or equivalent, (3) having ≥ 3 years of basketball training and competition experience, (4) having no diagnosed health conditions, medication for chronic medical conditions or injuries preventing/impeding exercise participation, (5) having no caffeine allergy or intolerance, (6) having a daily average self-reported consumption < 200 mg of caffeine (Beaumont et al., 2016), (7) not consuming any supplements, and (8) attaining ≥ 15% on a 3-point accuracy test (score ≥ 6 of 40 shots) during the familiarization trial.

Study Design

A double-blind, placebo-controlled, randomized design was used. Participants visited the laboratory and a sheltered outdoor basketball court on four occasions – one familiarization trial and three different conditions separated by at least one week: (i) placebo (maltodextrin) (Bulk™, Colchester, UK) and placebo (PP), (ii) caffeine (Bulk™, Colchester, UK) and placebo (CP), and

(iii) placebo and caffeine (PC). Caffeine or placebo doses were administered at 3 mg/kg BM in identical capsules prepared by two authors (ZST and RYYS). Prepared capsules were handed to an uninvolved third individual who randomized the allocation order (Urbaniak & Plous, 2013) before assigning an anonymous code to both sets to ensure blinding to both participants and investigators. Upon completion of data collection, the codes were unveiled to the investigators and the individual had no further involvement in the study.

Familiarization Trial

Height and weight were measured using electronic column scales (Seca 769, Seca GmbH & Co. Kg., Hamburg, Germany). The exercise protocol was described in detail to each participant (Scanlan et al., 2014) before they proceeded to complete the entire protocol (see Main Conditions below), without ingesting any pills.

Main Conditions

Participants abstained from alcohol and caffeine containing foods and beverages for at least 24 hours prior to each condition - the mean half-life of caffeine is reported to be 3 to 6 hours (Chia et al., 2017). All experimental conditions were performed in the morning. For standardization and ecological validity, two slices of bread slathered with coconut jam (approximately 31g carbohydrates, 3g fat, 6g protein and 180kcal) were provided for participants to consume for breakfast approximately 1-2 hours before departing to the laboratory.

The whole protocol lasted ~149 mins (Figure 2). Participants were initially provided with two unidentifiable gelatine pills containing either caffeine or placebo for consumption, with 60 minutes passive rest in the laboratory in-between ingestion of each pill (Time 1 and 2). Participants then moved to a nearby basketball court and completed a standardized 15-minute warm-up of basketball-specific drills and stretching (Stevanovic et al., 2019) before starting the Basketball Exercise Simulation Test (BEST) (Scanlan et al., 2014) and associated 3-point shooting protocol (Time 3). After finishing the BEST and shooting protocol (Time 4), a 15-minute cool-down static stretching routine was completed. Upon completion, blinding success was measured by asking participants and investigators to identify the condition the participant underwent (Time 5). A 7-item yes/no questionnaire was administered the morning following each condition for reporting of any side effects following caffeine consumption (Chia et al., 2017; Pallarés et al., 2013).

BEST and Shooting Protocol

Each main experimental condition consisted of four quarters. Each quarter involved 16 rounds of the BEST (each round to be completed within a maximum of 30 seconds) lasting 8 mins followed by a maximum of 2 mins to complete ten 3-point shots. Participants who completed each round of the BEST or 3-point shots ahead of time rested in the remaining period. There was 2 mins rest between the first and second and third and fourth quarters and 15 mins rest between the second and third quarters, replicating game demands under International Basketball Federation rules.

One round of the BEST requires participants to sprint, jog, run, jump, and shuffle (in a defensive stance position) for a total of 71.9 m (Scanlan et al., 2014). Vertical jump height, 6 m sprint performance, using timing gates (Swift, Lismore, Australia), and time taken to complete each round were recorded (Time 3 to 4; Figure 2). A Vertec device (Sports Imports, Hilliard, Ohio, US) was used to measure vertical jump height, adjusted 25.4 cm above each participant's standing maximum reach before the protocol began (Scanlan et al., 2014). Participants were instructed to standardize their jumping mechanics by: (1) jumping off with two feet and (2) reaching the Vertec device as high as possible with one arm. The shooting protocol consisted of two rounds of five 3-point shots - two from both corners at 180°, two from both wings at 45°, one from centre at 90° (Figure 3). Shot accuracy was recorded as 0 being a miss and 1 being a score.

Blood Sampling

Venous blood was collected from the antecubital veins of a subset of 10 participants (BD Vacutainer®, BD, New Jersey, US), 60 minutes after the first pill ingestion (Time 2) and after completing the cool-down (Time 5). Blood samples were left to clot for 30 minutes at room temperature before centrifugation at 1,163 x g for 15 minutes (Clay Adams™ Compact II Centrifuge, BD, New Jersey, US). The resultant serum was pipetted into cryogenic vials (Cryo.s™, Greiner Bio-One GmbH, Frickenhausen, Germany) and stored at -80°C for analyses.

Quantification of caffeine and related metabolites

Caffeine and its major metabolites (paraxanthine, theobromine and theophylline) were quantified via a targeted liquid chromatography-mass spectrometry (LC-MS) method. Firstly, serum samples were spiked with deuterated standards (0.05 μM final concentration), extracted with 200 μL acidified methanol (0.1% formic acid) and centrifuged for 10 minutes at 13,000 rpm at 4°C. The supernatant (200 μL) was collected and added into 200 μL of acetonitrile before undergoing centrifugation under the same conditions. The eventual supernatant was collected and stored at -80°C until ready for LC-MS analysis.

The extracts were analysed using a Waters Xevo TQ-S Triple Quadrupole MS system. Chromatographic separation was performed using a Waters Acquity UPLC HSS T3 column (2.1x150mm, 1.8μm) at 0.4 mL/min at 40°C. Mobile phases A and B were water (0.1% formic acid) and acetonitrile (0.1%), respectively. Gradient conditions were: 80%A (0 min), 80%A (0.5 min), 10%A (4 min), 10%A (6 min), 80%A (6.1 min) and 80%A (8 min). For detector conditions, capillary voltage was 1.0 kV, desolvation temperature was 450°C, source temperature was 150°C, gas cone flow was 150L/h and desolvation gas flow was 900 L/h. All metabolites were detected and quantified in multiple reaction monitoring mode in positive electrospray ionization mode. Paraxanthine and theophylline, having high structural similarity, share the same monoisotopic mass and retention time, and eluted as a single peak. We therefore combined quantification of these two compounds for analysis. Mass transitions and compound-dependent parameters were optimized using commercially authentic standards (Table 1). Calibration curves for quantification ranged from 1-200 ng/mL.

153 **Psychological Variables**

154 Perceived performance was measured using a 0-100 sliding scale; self-confidence, motivation,
155 and aggression using an 11-point Likert scale (0 = *not at all*; 10 = *very much*); and feeling and
156 felt arousal using 11-point (-5 = *very bad*; +5 = *very good*) and 6-point (1 = *low arousal*; 6 =
157 *high arousal*) Likert scales, respectively. These variables were assessed seven times (Times 1, 2
158 and 5 and end of each quarter; Figure 2) across each condition.

159

160 **Heart Rate and Rate of Perceived Exertion**

161 Heart rate (HR) (Polar® H7, Kempele, Finland) and rate of perceived exertion (RPE) (Borg,
162 1973) were measured immediately after the final shot in each quarter (Figure 2).

163

164 **Blood Glucose and Lactate**

165 Five blood glucose (BGlu) (Accu-Chek, Roche Diabetes Care GmbH, Mannheim, Germany) and
166 lactate (BLa) (Lactate Pro 2 LT-1730, Arkray Inc., Kyoto, Japan) measurements were recorded
167 throughout each condition (pre-exercise and after every quarter; Figure 2) via finger-prick
168 testing.

169

170 **Statistical Analysis**

171 Data were analyzed using SPSS v26.0 software (IBM Corp, Armonk, New York, US). One-way
172 repeated measures ANOVA was used to compare differences in environmental conditions and 3-
173 point shooting accuracy among the 3 conditions. Two-way repeated measures ANOVA were

used to compare differences across the 3 conditions and 4 quarters for secondary outcomes, HR and RPE, 3 conditions and 5 time points for BGlu and BLa, and 3 conditions and 7 time points for psychological variables. Greenhouse-Geisser correction was applied when the assumption of sphericity was violated. Holm-Bonferroni t-tests were used for post-hoc analyses when a significant interaction occurred. Pearson correlation analyses were employed to examine the association between pre- and post-caffeine metabolites with primary and secondary outcomes. Statistical significance was set at $p < 0.05$. Smallest worthwhile change (SWC) was calculated as 0.2×between-condition deviation for 3-point shooting accuracy and basketball performance parameters (Tan et al., 2020) to identify responders, non-responders, or negative responders to caffeine. The SWC comparisons compared caffeine conditions (CP and PC) with placebo (PP) and caffeine conditions alone (CP vs PC). Data are presented as mean ± standard deviation.

Results

Environmental Conditions

Testing was conducted under similar conditions of temperature (PP = 30.8 ± 2.8 °C; CP = 30.9 ± 2.4 °C; PC = 30.7 ± 2.0 °C; $p = 0.925$) and humidity (PP = 72.5 ± 10.8%; CP = 71.6 ± 7.5%; PC = 73.7 ± 8.0%; $p = 0.688$).

Three-point Shooting Accuracy

There was no significant difference in 3-point shooting score among conditions (PP = 12.33 ± 4.10; CP = 12.61 ± 2.81; PC = 11.67 ± 3.77; $p = 0.648$; Figure 4). Analyses based on a SWC of

0.69 (PP/CP), 0.78 (PP/PC), and 0.66 (CP/PC) indicated that more than half of participants were negative or non-responders in the two caffeine conditions versus placebo (Table 2).

Vertical Jump Height, Sprint Timing and BEST Completion Time

There was no significant interaction or main effects of condition or quarter on vertical jump height (Table 3). Analyses using SWC of 1.88 (PP/CP), 1.55 (PP/PC), and 1.91 (CP/PC) found no more than 50% (n = 9 of 18) of participants responded to either caffeine condition.

For 6 m sprint times, there was no significant interaction or main effect of condition but sprint times increased as the quarters progressed (Table 3) with post-hoc tests indicating all quarters significantly differing (all $p < 0.027$). Using SWC of 0.04 (PP/CP and PP/PC) and 0.03 (CP/PC) - with negative differences larger than SWC interpreted as responders due to the time measure - 11 participants were negative or non-responders on both caffeine conditions (PP/CP and PP/PC).

There were significant main effects of condition and quarter for BEST completion timing but no interaction. Post-hoc tests suggested that completion timing was slower on PP than both caffeine conditions (both $p = 0.054$) but not different between caffeine conditions ($p = 0.874$). BEST completion timing differed across all quarters (all $p < 0.001$) except quarter 3 and 4 ($p = 0.525$) (Table 3). Using SWC of 0.32 (PP/CP), 0.33 (PP/PC), and 0.31 (CP/PC), half to two-thirds of individuals responded to the caffeine conditions (CP = 9, PC = 12 of 18) compared with PP.

Caffeine Metabolites

One participant was excluded from the analysis as the caffeine concentration in the PP condition was larger than three standard deviations away from the mean. There was a significant interaction and main effects of time and condition for caffeine (all $p < 0.001$, Figure 5A) and paraxanthine and theophylline (all $p < 0.001$, Figure 5B). Post-hoc tests identified that both caffeine and paraxanthine and theophylline were significantly higher at Time 2 on the CP than PP or PC conditions (both $p < 0.05$). Between Time 2 and Time 5 caffeine decreased significantly on the CP condition and increased significantly on the PC condition (both $p < 0.05$) whereas paraxanthine and theophylline increased on the CP and PC conditions (both $p < 0.05$) but remained unchanged on the PP condition ($p > 0.05$). At Time 5, caffeine and paraxanthine and theophylline were significantly higher on both caffeine conditions than PP (both $p < 0.05$). Caffeine was significantly higher on PC than CP at Time 5 ($p < 0.05$). There was no main effect of condition or interaction for theobromine (Figure 5C), but a main effect of time was observed ($p = 0.007$) with theobromine increasing on the CP condition between Time 2 and Time 5. There was a negative correlation between BEST completion timing with pre- ($p = 0.028$, $r = -0.422$) and post-paraxanthine and theophylline ($p = 0.016$, $r = -0.459$). Sprint timing was positively associated with pre- ($p = 0.004$, $r = 0.539$) and post-theobromine ($p = 0.018$, $r = 0.453$).

Psychological Variables

There were no significant interactions or main effects of condition for the six psychological variables measured (all $p > 0.180$). A main effect of time was observed only for felt arousal ($p < 0.001$) (Figure 6A) and aggression ($p < 0.001$) (Figure 6B), with pre-exercise measures (times 1

and 2) significantly lower than all exercise and post-exercise time measures (all $p < 0.028$) for both variables.

Heart Rate and Rate of Perceived Exertion

Heart rate data from two participants were excluded from analysis as sensors were unable to accurately measure the heart rate upon completion of exercise in two separate conditions (data two to three standard deviations less than the mean value of other participants). There was no main effect of condition ($p = 0.860$) or interaction effect for HR ($p = 0.885$) but a main effect of quarter was observed ($p < 0.001$) with higher HR in Q2 (mean increase PP = 5.0 ± 4.7 bpm, CP = 7.7 ± 7.0 bpm, PC = 4.0 ± 8.3 bpm, $p < 0.001$) and Q4 (mean increase PP = 6.5 ± 8.1 bpm, CP = 6.4 ± 13.4 bpm, PC = 5.4 ± 7.4 bpm, $p = 0.006$) than Q1. Similarly, for RPE there was no main effect of condition ($p = 0.137$) or interaction ($p = 0.538$). However, RPE increased significantly ($p < 0.001$) across all quarters except between Q2 and Q3 (data not shown).

Blood Glucose and Lactate

No significant interactions were observed for BGlu ($p = 0.135$) or BLa ($p = 0.71$). For BGlu, there was no main effect of quarter ($p = 0.089$) but a significant main effect of condition ($p = 0.042$), with higher BGlu in CP than PP (mean difference = 0.34 ± 0.57 mmol/L, $p = 0.044$) only. A main effect of condition ($p = 0.018$) was observed for BLa, with higher BLa in CP (mean difference = 1.31 ± 2.86 mmol/L, $p = 0.029$) and PC (mean difference = 1.17 ± 2.79 mmol/L, $p = 0.038$) than PP but no difference between caffeine conditions (mean difference = 0.14 ± 3.01 mmol/L, $p = 0.777$). There was also a main effect of quarter ($p = 0.015$) for BLa, with

significantly higher BLa in Q1 than Q3 (mean increase PP = 1.89 ± 3.14 mmol/L, CP = 0.93 ± 2.59 mmol/L, PC = 1.84 ± 2.94 mmol/L, $p = 0.002$).

Side Effects and Blinding

Muscular pain was reported in all conditions (PP = 22.2%, CP = 27.8%, PC = 16.7%, $n = 18$). Gastrointestinal problems, nervousness, increased irritability, and headache were reported by 1 participant in all conditions. Less than half of the 54 conditions were accurately predicted by the participants (mean = 46.3%) or investigators (mean = 38.0%).

Discussion

This study is the first to compare the effects of caffeine ingestion timing, either 1 hour or immediately pre-exercise (warm-up), on 3-point shooting accuracy in basketball (Guest et al., 2021; Shen et al., 2019). Irrespective of ingestion timing, no effect of caffeine on 3-point shooting was observed. Our systematic and standardized protocol improves on previous investigations where the number of 3-point shots taken, either in simulated game play or from around the 3-point line, was unequal (although not necessarily statistically different) between caffeine and placebo conditions (Abian-Vicen et al., 2014; Puente et al., 2017). Moreover, we included a standardized simulated basketball task before shooting for improved ecological validity as the shooting accuracy of basketball players can be impacted by fatigue (Mulazimoglu et al., 2017).

Several reasons might explain why shooting accuracy was unaffected by caffeine. Potentially 3 mg/kg was insufficient to improve accuracy but this dose can improve other aspects of game play performance in basketball (Chia et al., 2017) and has improved shooting accuracy in one existing study (Puente et al., 2017), so this seems unlikely. Secondly, whilst caffeine administration was based on current knowledge of its pharmacokinetics, variations in *CYP1A2* - the gene which encodes for the enzyme accountable for 95% of caffeine metabolism in the body – results in two types of caffeine metabolisers. Individuals who are C allele carriers metabolise caffeine slower than AA genotypes and consequently may experience lower or no ergogenic effects (Pickering & Kiely, 2019). However, a key limitation is that we were unable to measure genotype and the standard deviations (Figure 5) of caffeine and paraxanthine and theophylline in each condition were not large as might be expected if any genotype effect were present. Another key limitation is that the metabolomics data represent only half the participants and the measures taken were only pre- and post-exercise. Thus, we cannot confirm when peak concentrations occurred and there exists considerable variability around both the peak concentration (Guest et al., 2021) and half-life of caffeine (Chia et al., 2017). Task sensitivity is another possible reason for why caffeine did not affect shooting. To try to offset this, participants needed to score a minimum of 15% of shots during the familiarisation trial for inclusion. Finally, caffeine tolerance increases in regular consumers. Our participants self-reported consuming 0-193 mg of caffeine per day but these are estimates because of varying levels of caffeine in many drinks (Chia et al., 2017).

A unique feature of this study was the inclusion of vertical jumps and linear sprints as part of a continuous fatiguing protocol simulating game demands, rather than as part of a battery of fitness

tests with standardized rest periods as per previous investigations. The effects of caffeine on vertical jump are equivocal. Significant improvements in countermovement or repeated jump height of basketball players have been reported in four studies employing 3 (Abian-Vicen et al., 2014; Puente et al., 2017; Stojanović et al., 2021) or 6 mg/kg (Raya-González et al., 2021) of caffeine, but one study utilizing a ~2.3 mg/kg caffeinated chewing gum (Filip-Stachnik et al., 2022) and two using 3 mg/kg caffeine in capsules (Stojanović et al., 2019; Tucker et al., 2013) did not observe improvements. Timings of a single 20 m sprint and 5 × 30 m repeated shuttle sprint improved when 6 mg/kg of caffeine was provided in a drink to 14 professional male basketball players (Raya-González et al., 2021) but sprints of between 5-20 m in length were unaffected in the present study or four previous studies utilizing only 3 mg/kg (Filip-Stachnik et al., 2022; Scanlan et al., 2019; Stojanović et al., 2019; Stojanović et al., 2021). Thus, a higher caffeine dose may be needed to improve sprint performance.

A novel finding was that both caffeine conditions improved BEST completion times. Caffeine can reduce pain perception and RPE to enhance exercise capacity (Pickering & Kiely, 2019) but RPE did not differ among conditions in our study. Possibly caffeine afforded participants the ability to complete the BEST more quickly at the same relative pain level, although other relevant psychological measures such as motivation and felt arousal were unchanged. Two previous studies found no effect of 3 mg/kg of caffeine on accelerometer measures (Puente et al., 2017) or distance covered at different speeds (Raya-González et al., 2021) during simulated basketball matches. However, the non-standardised nature of these matches makes comparisons difficult whereas the standardised BEST protocol implemented in this study may have more easily detected a difference in completion time with caffeine. BEST completion timings were

also negatively related to pre- and post-paraxanthine and theophylline. Paraxanthine is considered a more potent adenosine-receptor antagonist than caffeine (Benowitz et al., 1995) and known to affect locomotor activity (Davenport et al., 2020). Pre-exercise caffeine consumption (CP) increased BGlu overall compared with PP and BLa was higher on both caffeine interventions than PP. These observations may also explain the improvement in BEST completion times, with circulating glucose an important contributor to energy supply during high intensity exercise (Adams, 2013) and blood lactate an indicator of anaerobic glycolysis/glycogenolysis (Stojanović et al., 2018).

Conclusions

Manipulating ingestion timing of 3 mg/kg of caffeine from 1 hour before to immediately pre-exercise did not improve 3-point shooting accuracy, vertical jump height and sprint timings. From a novel and practical perspective, our data demonstrate that 3mg/kg caffeine can influence work completed in basketball simulated exercise without side effects, irrespective of ingestion timing.

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Protocol: The study was prospectively registered with the Australian New Zealand Clinical Trials Registry (registration number ACTRN12621001308886). Data related to this publication will be placed in the Data Repository of the Nanyang Technological University National Institute of Education (Singapore) and made available upon reasonable request to the Corresponding Author (SFB).

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Tables

Table 1. Mass transitions and compound-dependent parameters.

Compound	Mass transition (m/z)	Retention time (min)	Fragmentation voltage (V)	Collision energy (eV)
Caffeine	195.1 → 138.2	1.63	30	20
Caffeine-d9	204.2 → 144.0	1.61	30	20
Paraxanthine and Theophylline	181.0 → 124.2	1.26	30	20
Theophylline-d6	187.3 → 127.0	1.28	30	20
Theobromine	181.1 → 137.8	1.15	30	20
Theobromine-d6	187.3 → 144.2	1.15	30	20

474 **Table 2.** Individual analyses of responses to caffeine ingestion for 3-point shooting accuracy relative to

475 the smallest worthwhile change.

Participant	PP/CP difference	SWC	PP/PC difference	SWC	CP/PC difference	SWC
1	8	↑	-1	↓	-9	↓
2	-4	↓	-1	↓	3	↑
3	-5	↓	-4	↓	1	↑
4	5	↑	0	↔	-5	↓
5	0	↔	-2	↓	-2	↓
6	0	↔	6	↑	6	↑
7	8	↑	3	↑	-5	↓
8	-2	↓	-4	↓	-2	↓
9	4	↑	7	↑	3	↑
10	-2	↓	-9	↓	-9	↓
11	1	↑	-1	↓	-2	↓
12	3	↑	2	↑	-1	↓
13	0	↔	-2	↓	-2	↓
14	2	↑	-4	↓	-6	↓
15	0	↔	0	↔	0	↔
16	-3	↓	1	↑	4	↑
17	-2	↓	4	↑	6	↑
18	-8	↓	-5	↓	3	↑

476

477 **Table 3.** Mean ± SD of vertical jump height (cm), 6 m sprint timings (s) and BEST completion timings (s) measured across three conditions (PP, CP,
478 and PC) and four quarters (Q1 to Q4).

Conditions	Vertical Jump Height (cm)				3 x 4 repeated measures ANOVA outcomes					
	Q1	Q2	Q3	Q4	Interaction		Condition		Quarter	
					p	η^2_p	*p*	η^2_p	*p*	η^2_p
PP	62.8 ± 8.0	62.5 ± 7.8	62.2 ± 7.4	62.9 ± 7.8						
CP	63.7 ± 11.6	63.9 ± 11.9	63.4 ± 10.8	64.6 ± 10.4	0.622	0.032	0.582	0.026	0.083	0.122
PC	63.0 ± 8.3	63.5 ± 8.8	63.6 ± 7.7	64.8 ± 8.8						
	Sprint Timing (s)				3 x 4 repeated measures ANOVA outcomes					
	Q1	$Q2^{1}$	$Q3^{12}$	$Q4^{123}$	Interaction		Condition		Quarter	
					p	η^2_p	*p*	η^2_p	*p*	η^2_p
PP	1.58 ± 0.18	1.69 ± 0.21	1.75 ± 0.23	1.78 ± 0.21						
CP	1.60 ± 0.19	1.68 ± 0.17	1.69 ± 0.15	1.73 ± 0.14	0.134	0.106	0.493	0.041	**<0.001**	0.795
PC	1.56 ± 0.15	1.68 ± 0.19	1.73 ± 0.21	1.74 ± 0.23						
	BEST Completion Timing (s)				3 x 4 repeated measures ANOVA outcomes					
	Q1	$Q2^{1}$	$Q3^{12}$	$Q4^{12}$	Interaction		Condition		Quarter	
					p	η^2_p	*p*	η^2_p	*p*	η^2_p
PP	25.6 ± 1.9	26.4 ± 1.7	26.8 ± 1.7	26.9 ± 1.5						
CP_a	25.0 ± 1.5	25.9 ± 1.5	26.1 ± 1.7	26.3 ± 1.7	0.598	0.043	**0.031**	0.185	**<0.001**	0.75
PC_a	24.9 ± 1.6	25.7 ± 1.9	26.4 ± 1.9	26.4 ± 1.6						

479 **a** represents significant difference between conditions PP with PC and CP. **1** represents significant difference between quarters 1 with quarters 2,
480 3, and 4; **2** represents significant difference between quarters 2 with quarters 3 and 4; **3** represents significant difference between quarters 3 and
481 4. Bolded values indicate statistically significant difference ($p < 0.05$).

Figure Legends

Figure 1: CONsolidated Standards of Reporting Trials (CONSORT) flow diagram (Moher et al., 2010).

Figure 2: Schematic of the study procedure for one condition. Participants ingested a first pill (#1 - caffeine or placebo) at Time 1 before resting for 60 minutes and then ingesting a second pill (#2) at Time 2. After a 15-min warm-up, participants completed four 8-min quarters of the Basketball Exercise Simulation Test (BEST) (Scanlan et al., 2014), with a 2-min shooting protocol at the end of each quarter (Time 3-4). After a standardized cool-down, post-measure data were collected (Time 5).

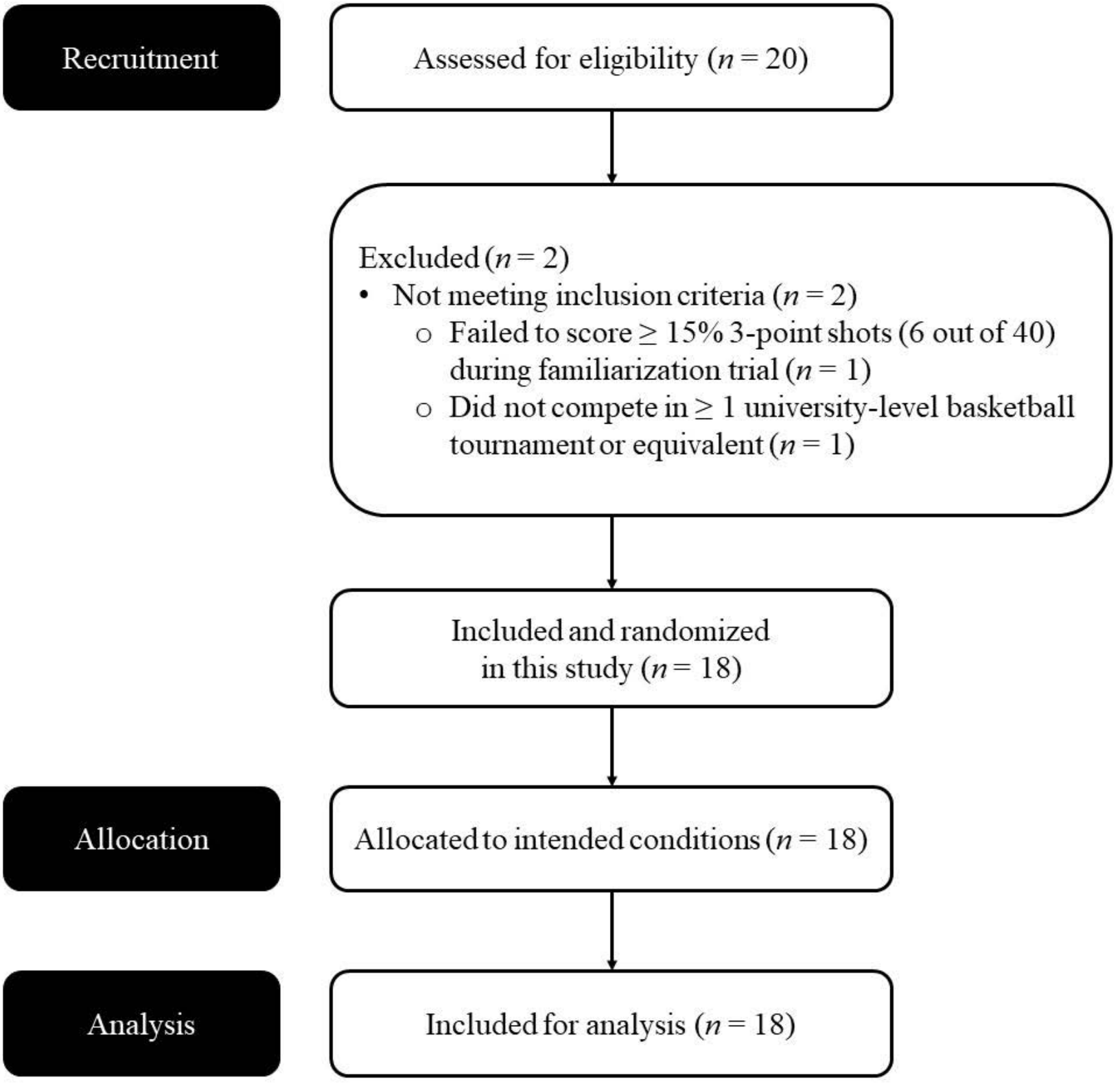
Figure 3: Shooting protocol. Each participant started from the half-court line, picked up a basketball and dribbled to the first spot marked by 1 before performing a 3-point shot. Participant ignored any rebound, ran back to the half-court line to pick up another basketball and proceeded to spots 2, 3, 4 and 5. Each participant completed two rounds totalling ten 3-point shots altogether.

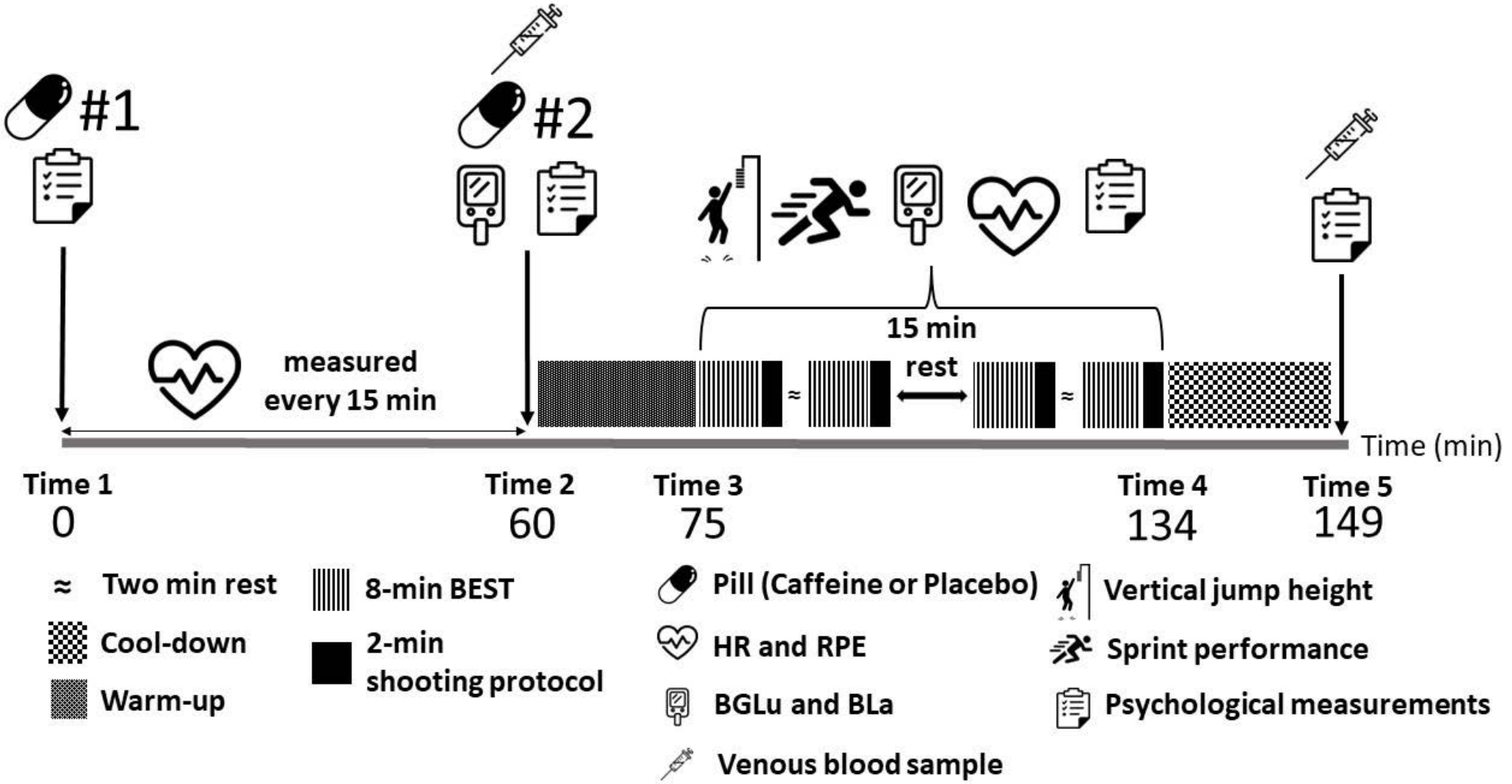
Figure 4: Means ± SD and individual total 3-point scores of three conditions (PP, placebo and placebo; CP, caffeine and placebo; and PC, placebo and caffeine.

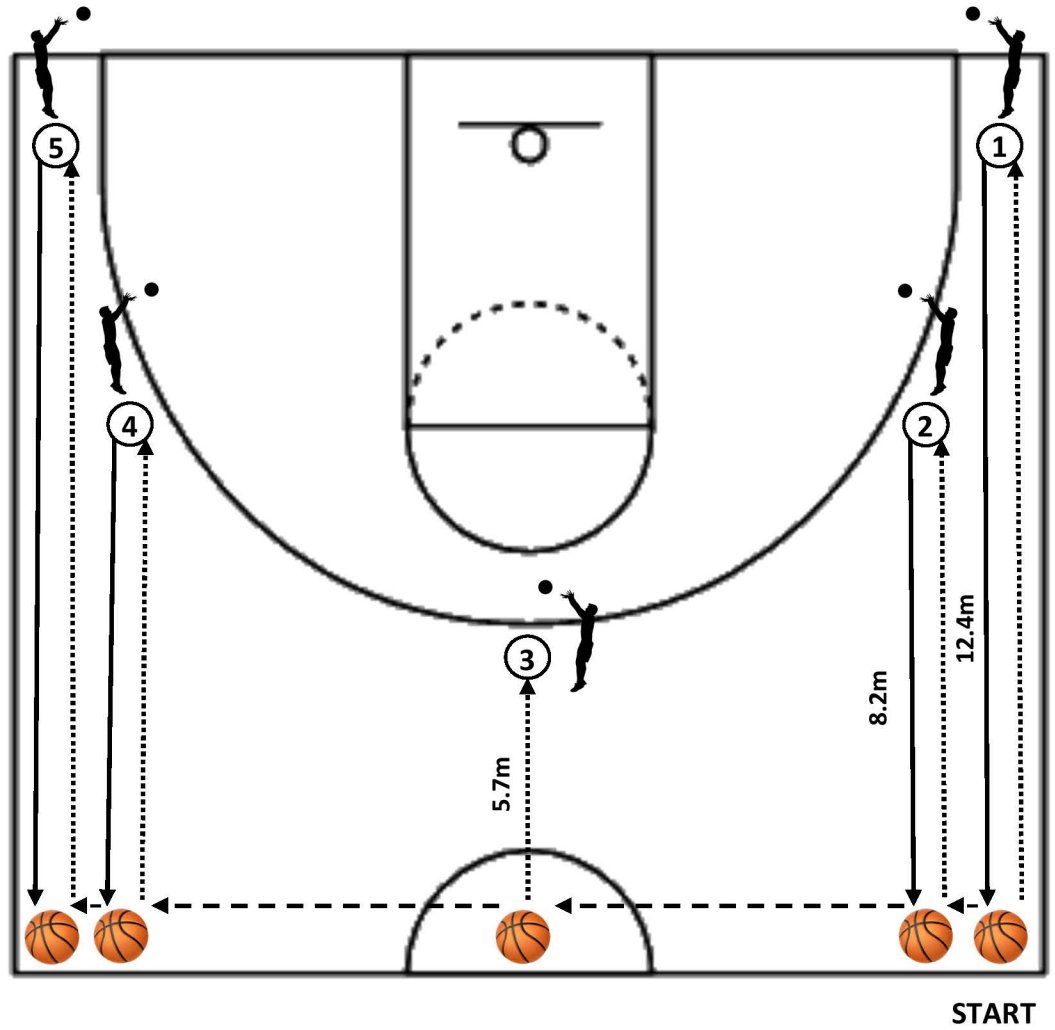
Figure 5: Mean serum metabolite concentrations (ng/ml) for A) caffeine, B) paraxanthine and theophylline, and C) theobromine across three conditions (PP, CP, PC) measured at Time 2 (60^{th} min) and Time 5 (149^{th} min). For Figure 5A, a denotes statistical significance ($p < 0.05$) for CP Time 2 *vs.* CP Time 5, PP Time 2 and PC Time 2; b denotes $p < 0.05$ for PC Time 2 *vs.* PC Time 5; and c denotes $p < 0.05$ between PP, PC and CP at Time 5. For Figure 5B, d denotes $p < 0.05$ for CP Time 2 *vs.* CP Time 5, PP Time 2 and PC Time 2; e denotes $p < 0.05$ for PC Time 2 *vs.*

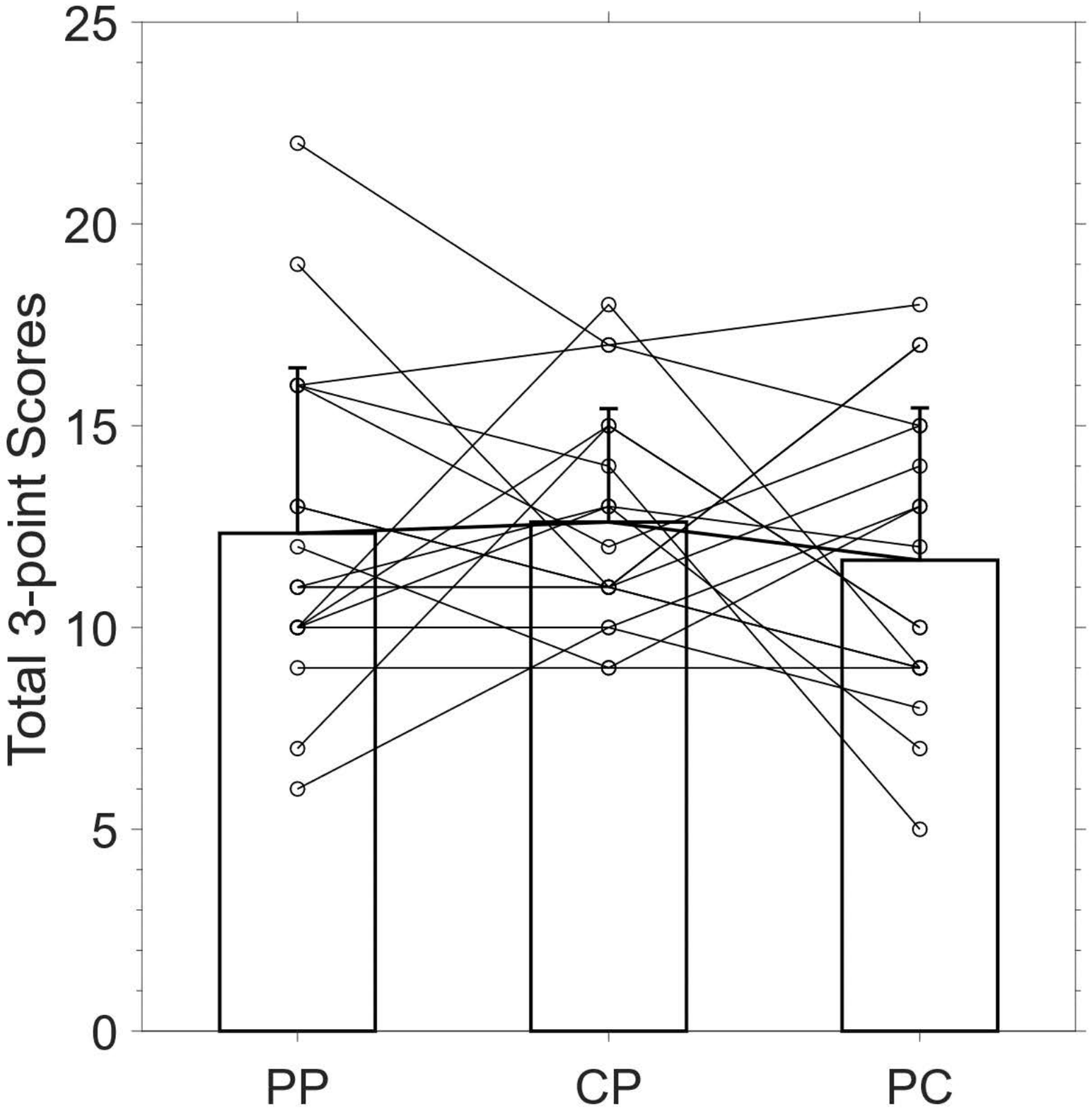
504 PC Time 5; and f denotes $p < 0.05$ for PP *vs.* PC and CP at Time 5. For Figure 5C, g denotes $p <$
505 0.05 CP Time 2 *vs.* CP Time 5.

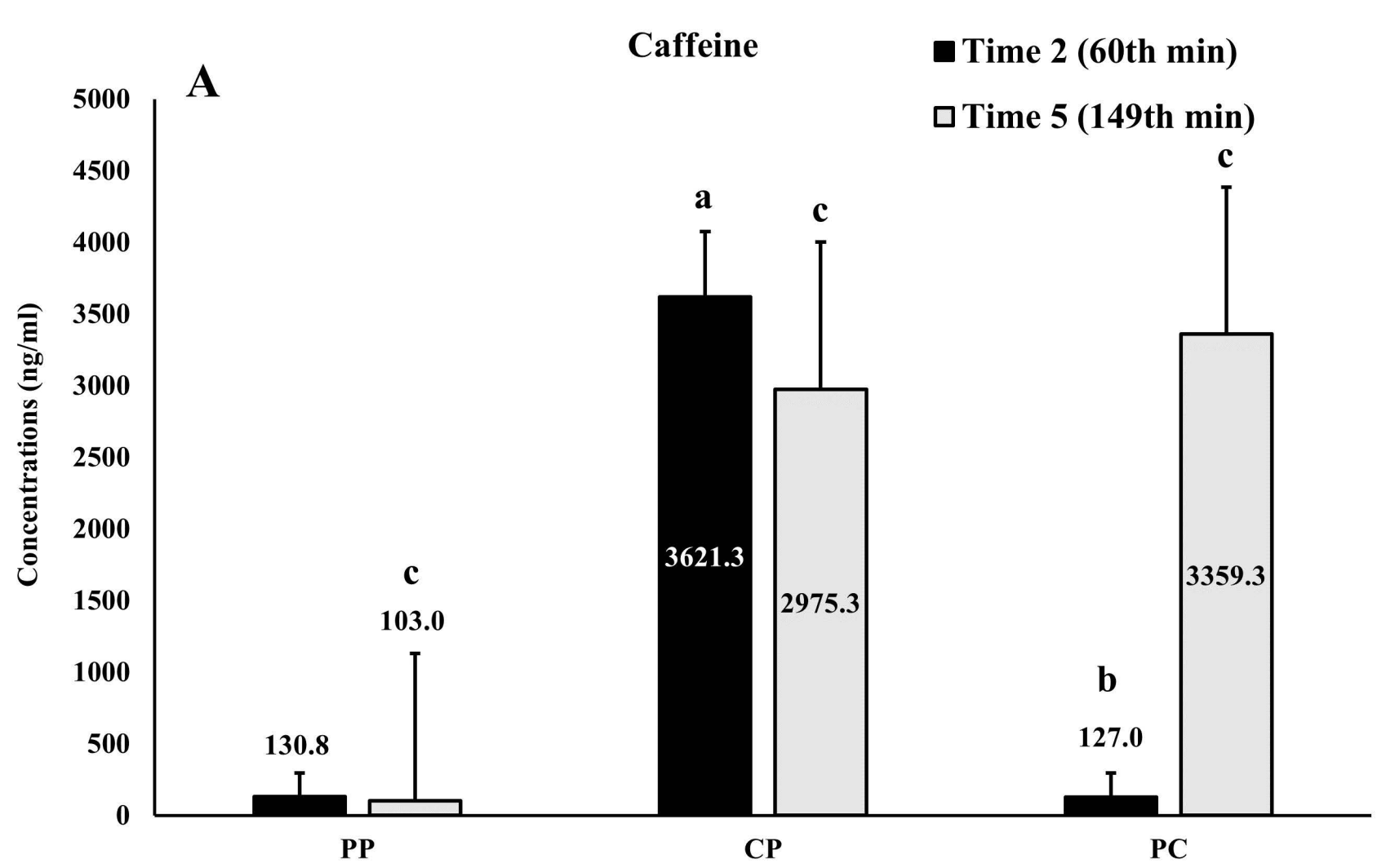
506 **Figure 6:** Mean ± SD of A) Felt arousal scale ratings and B) aggression scale ratings measured
507 across three conditions and time. * denotes statistical significance ($p < 0.01$) for 0 min with 60^{th}
508 to 149^{th} min while ** denotes statistical significance ($p < 0.01$) for 60^{th} min with 87^{th} and 124^{th}
509 minute for felt arousal. α denotes statistical significance ($p < 0.01$) for 0 min with 60^{th} to 149^{th}
510 min while β denotes statistical significance ($p < 0.01$) for 60^{th} min with 75^{th} to 124^{th} min for
511 aggression.

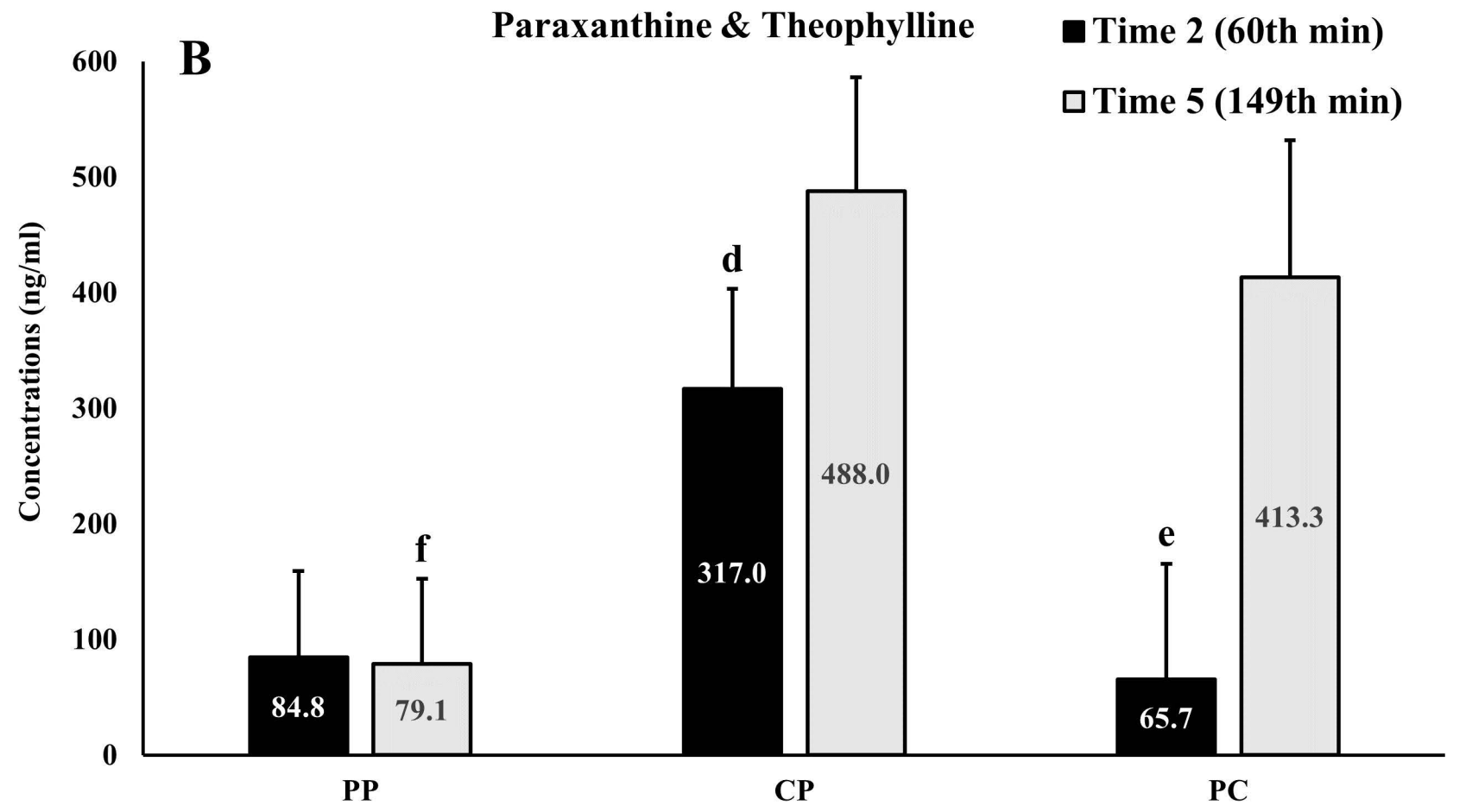

Recruitment
Assessed for eligibility (n = 20)
Excluded (n = 2)
• Not meeting inclusion criteria (n = 2)
o Failed to score ≥ 15% 3-point shots (6 out of 40) during familiarization trial (n = 1)
o Did not compete in ≥ 1 university-level basketball tournament or equivalent (n = 1)
Included and randomized in this study (n = 18)
Allocation
Allocated to intended conditions (n = 18)
Analysis
Included for analysis (n = 18)

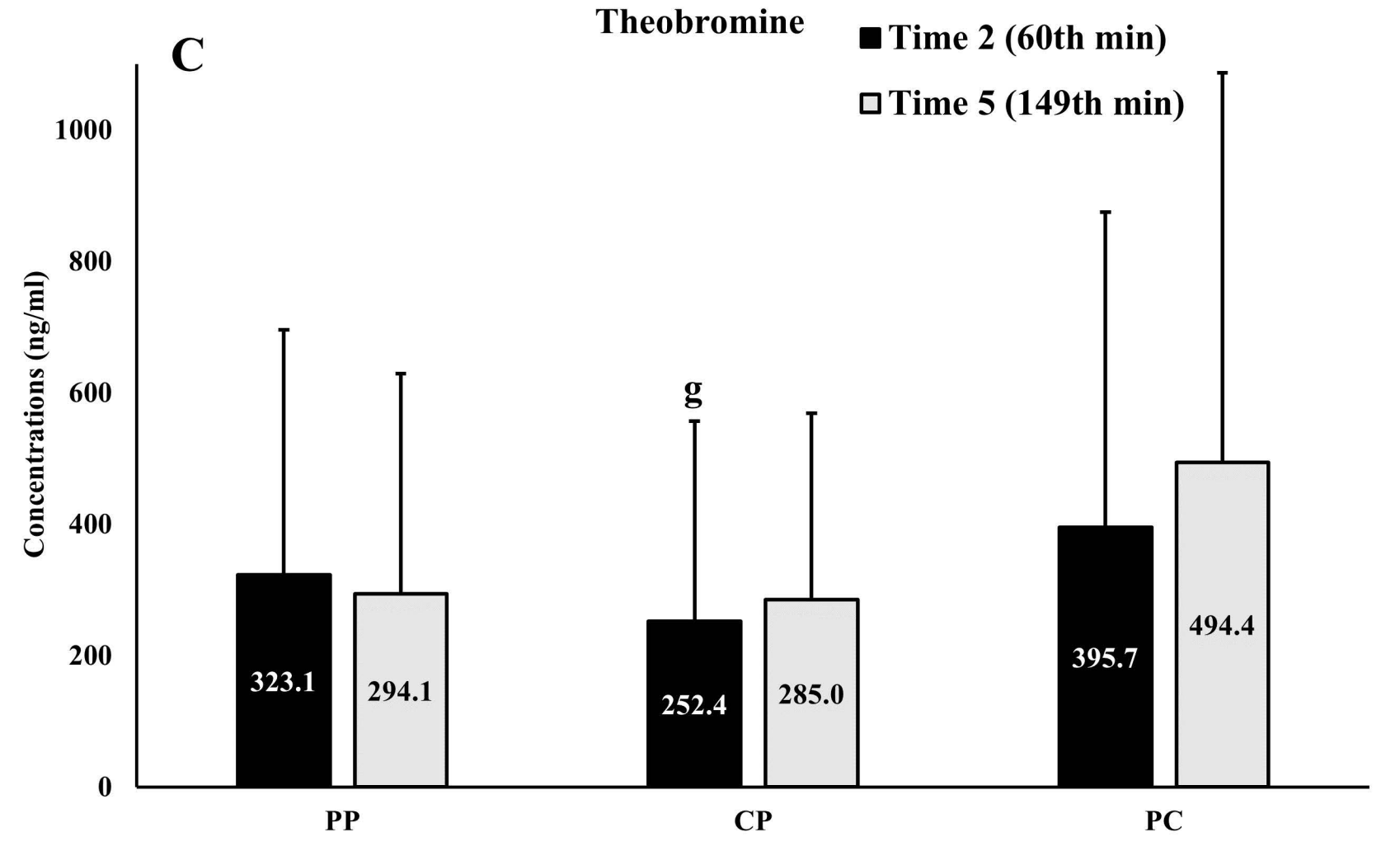

#1
#2
measured
every 15 min
15 min
rest
Time (min)
Time 1
0
Time 2
60
Time 3
75
Time 4
134
Time 5
149
≈ Two min rest
Cool-down
Warm-up
8-min BEST
2-min
shooting protocol
Pill (Caffeine or Placebo)
HR and RPE
BGLu and BLa
Venous blood sample
Vertical jump height
Sprint performance
Psychological measurements

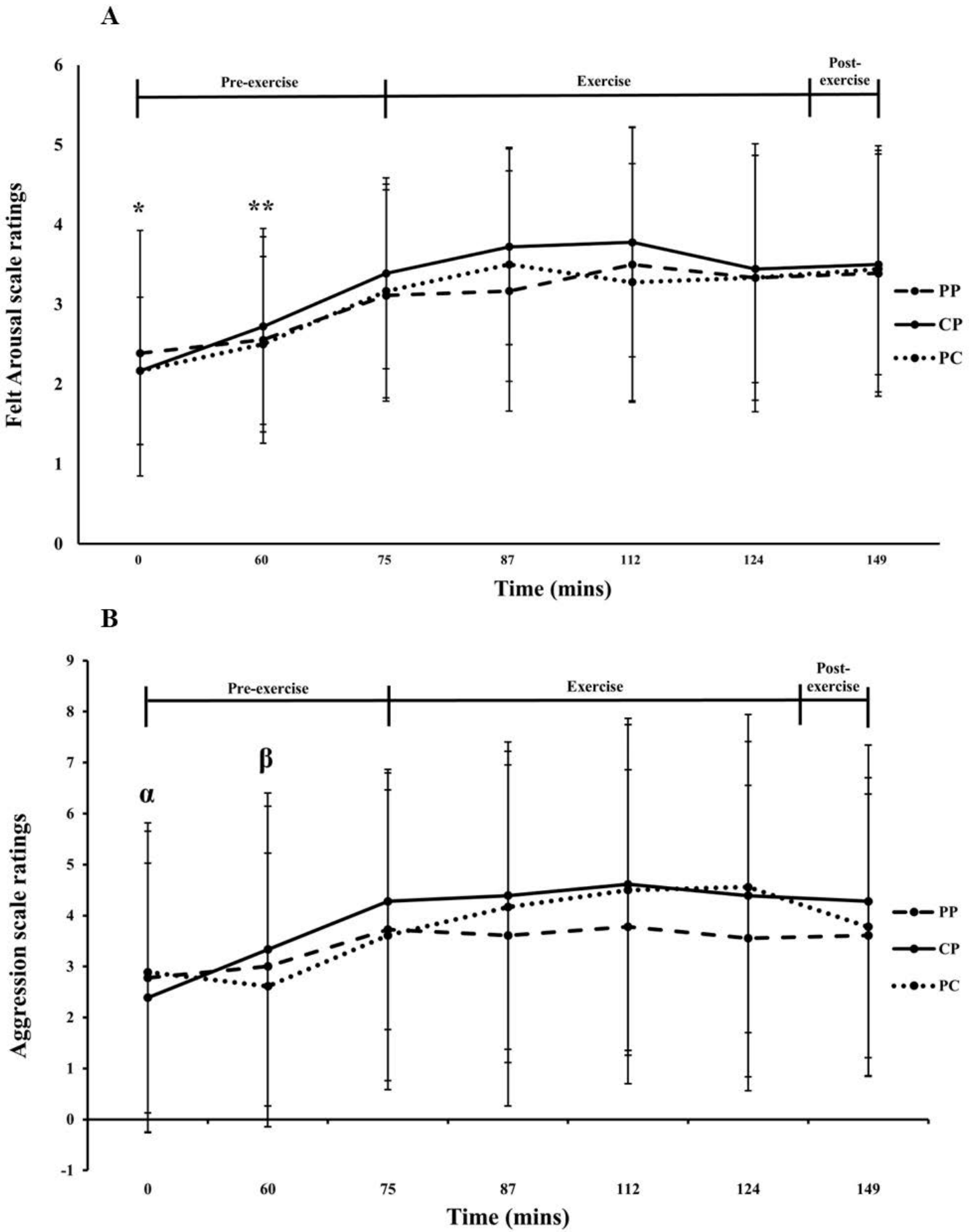

5
1
4
2
3
8.2m
12.4m
5.7m
START


25
20
15
10
5
0
Total 3-point Scores
PP
CP
PC


A
Caffeine
Time 2 (60th min)
Time 5 (149th min)
Concentrations (ng/ml)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
a
c
c
b
130.8
103.0
3621.3
2975.3
127.0
3359.3
PP
CP
PC


B
Paraxanthine & Theophylline
Time 2 (60th min)
Time 5 (149th min)
Concentrations (ng/ml)
600
500
400
300
200
100
0
d
f
e
84.8
79.1
317.0
488.0
65.7
413.3
PP
CP
PC


C
Theobromine
Time 2 (60th min)
Time 5 (149th min)
Concentrations (ng/ml)
1000
800
600
400
200
0
g
323.1
294.1
252.4
285.0
395.7
494.4
PP
CP
PC


A
Pre-exercise
Exercise
Post-exercise
Felt Arousal scale ratings
Time (mins)
PP
CP
PC
*
**
B
Pre-exercise
Exercise
Post-exercise
Aggression scale ratings
Time (mins)
PP
CP
PC
α
β

PRESENT 2020 (PROPER REPORTING OF EVIDENCE IN SPORT & EXERCISE NUTRITION TRIALS)

CHECKLIST OF INFORMATION TO INCLUDE WHEN REPORTING RESEARCH IN SPORT NUTRITION & EXERCISE METABOLISM*

Section	Item	Checklist	Line (if applicable)
Title			
	1a	State the independent (groups/conditions) and dependent (outcome) variables	Y
	1b	Identify the study population or case	Y
Abstract			
	2a	Specify the research design, methods and characteristics of study population	Y
	2b	Report a balanced account of the results and cite actual data	Y
	2c	Restrict conclusions to measured variables, without speculation or unsupported recommendations	Y
Introduction			
	3a	Present a scientific rationale based on an objective review of available evidence	6-34
	3b	State the aims, objectives, research questions and/or hypotheses	36-42
Methods			
-Ethics	4	Provide details of ethical approval (citing conduct of human research in accordance with the Declaration of Helsinki)	46-8
-Design	5	Summarize the research design (e.g. parallel trial/cross-over, randomized, counterbalanced, blinding, observational)	63
-Sampling	6a	List the eligibility (inclusion/exclusion) criteria and sampling method	52-60
	6b	Characterize the study sample (e.g. demographics, anthropometry, lifestyle)	52-3
	6c	Report the setting/location and periods of recruitment and data collection	63-4
	6d	Justify the sample size (presenting the selected target effect size and error variances to replicate sample size estimates)	50-2
-Interventions‡	7	Detail all aspects of the groups/conditions (considering the need to verify the composition of ingested substances)	65-8, 88-99, Figure 2
-Measurements	8a	Define the pre-specified primary, secondary and/or mechanistic outcome variables	36-9
	8b	Rationalize the selection of test protocols, considering validity and reliability (e.g. coefficient of variation, familiarization)	102-20
	8c	Justify the smallest worthwhile effect or minimal clinically important difference	180-84

-Randomization	9	Detail the exact mechanisms of generating and concealing the random allocation sequence	68-72
- Blinding[‡]	10	Document whether participants and/or researchers were aware of allocation (e.g. exit questionnaire)	68-72, 95-7
-Standardization	11	Describe within- and between-participant controls (e.g. replication/reporting of diet, physical activity, sleep, menstrual cycle)	81-6
-Order Effects	12	Detail control of systematic influences of serial measurements (e.g. sequence effect in analysis model, wash-out interval)	65
-Statistics	13a	Specify the contrast for primary inferences (i.e. relative to the appropriate control, not changes from baseline in each group/condition)	171-76
	13b	Clearly distinguish and fully justify any unplanned, interim or exploratory sub-group analyses	NA
	13c	Describe any adjustments for violated statistical assumptions and for relevant covariates (e.g. baseline measures)	NA
Results			
-Participant Flow	14a	Report the sample size at each phase from recruitment to analysis (with reasons for losses and exclusions)	52-3, Figure 1, 123, 217-8, 242-4
	14b	Ensure data analysis matches research design, avoiding data pooling across groups/conditions (i.e. pseudoreplication)	NA
-Outcomes	15a	Report *SI* units and report measures of central tendency, variability and effect size/precision (confidence intervals)	187-261, Figures 4-6, Table 2-3
	15b	Report individual data/responses (e.g. draw figures showing the raw data in each group/condition)	Figures 4-6, Tables 2-3
	15c	Document all relevant harms and unintended consequences observed	264-6
Discussion			
	16a	Present an objective and balanced interpretation of the observed data within the context of existing evidence	270-334
	16b	Consider the applicability and/or practical relevance of the research findings (e.g. external validity)	339-41
	16c	Acknowledge strengths and limitations of the research relevant to accurate interpretation (e.g. internal validity)	270-334
Other			
-Disclosures	17	State any relevant relationships (e.g. financial, technical, material support)	351-57

-*Protocol*	18	Identify any publicly-registered or published protocol (explaining any deviations)	358-59

**Adapted from the 2010 CONSORT checklist for reporting randomized controlled trials and can be used in conjunction with the associated paper that expands on each item.*

‡Items 7 (Interventions) and 10 (Blinding) are relevant for experimental research, including single/double blind contrasts of nutritional supplements.