

## Article

# One Week of Low or Moderate Doses of Caffeinated Coffee Consumption Does Not Induce Tolerance to The Acute Effects of Caffeine on Sprint Performance

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**Abstract:** Habituation to acute performance responses of caffeine intake is still an ongoing debate. The aim of this study was to evaluate the effects of acute and 1 week consumption of caffeinated coffee on intermittent sprint cycling performance (12x4s with 90s active recovery) (ISP). Twenty four male team-sport players randomly divided into 3 groups (8 athletes for each): 0.16 gr/kg decaffeinated coffee ingestion in a day for 1 week (PLAGROUP), 0.08 caffeinated + 0.08 decaffeinated coffee (providing 3 mg/kg caffeine) (0.16 gr/kg in total) (LOWGROUP) and 0.16 gr/kg caffeinated coffee (providing 6 mg/kg caffeine) (MODGROUP). In a randomized and double-blind design, participants underwent three test session: At the first two test sessions, ISP test was performed with acute ingestion of decaffeinated coffee (PLA) or 6 mg/kg of caffeine provided by coffee (FIRSTCAF) to test acute effects of caffeine intake. At the third test session, following to 1 week of coffee consumption, to test if tolerance develops, ISP was performed with ingestion 6 mg/kg of caffeine provided by coffee (SECONDCAF). A 2-way repeated measures ANOVA showed that although average peak ( $p=0,39$ ;  $\eta^2 =0,13$ ) and average mean ( $p=0,11$ ;  $\eta^2 =0,15$ ) power of total 12 sprints during ISP test were not statistically different between 1 week consumption groups, FIRSTCAF and SECONDCAF significantly increased peak power ( $p=0,01$ ;  $\eta^2 =0,44$ ) and mean power ( $p=0,01$ ;  $\eta^2 =0,46$ ) in the first three sprints compared to PLA in all consumption groups. It appears that no tolerance was developed in 1 week consumption of 3 or 6 mg/kg/day of caffeine provided by coffee.

**Keywords:** habituation; team sport; athlete; ergogenic aid; supplements.

## 1. Introduction

In team sports, it is a requirement having a high degree of intermittent sprint performance (ISP) as determinant of the success (Girard et al., 2011). Apparently, any supplement use that can improve ISP in team sports may contribute winning in competition. Of these, caffeine (CAF) is most commonly administered prior to and during

a game (Tallis et al., 2021). The acute intake of CAF doses of 3-6 mg/kg of body mass has been well established to improve performance in various exercise modalities including aerobic (Southward et al., 2018), anaerobic activities (Davis and Green, 2009), muscle performance such as strength (Grgic et al., 2020), muscular endurance (Flip-Stachnik et al., 2021), maximum power



(Dominguez et al., 2021) and intermittent sprint performance (Mohr et al., 2011; Glaister et al., 2019). On the other hand, it has been still reported to be ineffective on some performance parameters (Clarke et al., 2016; Trexler et al., 2016). Two main mechanisms have been proposed that exerts its effect centrally via adenosine A<sub>1</sub> and A<sub>2A</sub> receptors antagonism (Davis and Green, 2009) may increase in neurotransmitter release and peripherally potentiate the Na<sup>+</sup>/K<sup>+</sup> pump activity to enhance excitation-contraction coupling (Mohr et al., 2011).

One consideration with the use of CAF, particularly with anhydrous form, is the possibility of an overdose. High doses of CAF is known to cause various side effects such as tachycardia, insomnia and headache (Pallares et al., 2013). This risk can be minimized by using coffee, most concentrated and largest dietary source of caffeine preferred by athlete prior to competition while still providing performance benefits (Desbrow and Leveritt, 2006). To date, coffee was shown to be effective by most (Hodgson et al., 2013; Richardson et al., 2016; Clarke et al., 2019; McLellan and Bell, 2004) but not all studies (Marques et al., 2018; Karayigit et al., 2021). Vast majority of the aforementioned studies in the literature focused on aerobic or resistance exercise, only two studies examined the effect of coffee on sprint performance. Trexler et al., (2016) reported that ingestion of coffee providing 303 mg of CAF before 30 minutes testing attenuated the reductions in peak and total power during repeated sprint cycling in trained males. However, caffeinated coffee was compared statistically to placebo beverage with noncaloric flavoring rather than decaffeinated coffee which may constitute expectancy effect (Shabir et al., 2018). Further, Clarke et al., (2016) demonstrated that 3 mg/kg of CAF provided by coffee did not improved repeated sprint cycling performance in untrained males and suggested for future studies to assess habitual caffeine intake and conduct with more trained participants. In this regard, it is

a need to investigate the effects of caffeinated coffee, by comparing with the decaffeinated coffee to avoid expectancy effect, on sprint performance in trained athletes.

Chronic CAF intake was speculated to influence the concentration and activity of A<sub>1</sub> and A<sub>2A</sub> receptors that may affect ergogenic magnitude of acute caffeine intake (Pickering and Kiely, 2019). Early studies showed acute caffeine intake was less effective in participants already habituated (>300 mg/day) compared to individuals with very low daily caffeine usage (Bell and McLellan, 2002). Similar habituation was also shown by Beaumont et al., (2017) in which chronic 3 mg/kg of CAF intake developed tolerance to the acute 3 mg/kg of CAF on 30 min time trial performance. Questions remained to be solved whether higher doses of acute CAF intake (6 mg/kg) overcome tolerance effect of lower doses ( $\leq$  6 mg/kg) consumption. Supportively, habituation was described as “myth” by Gonçalves et al., (2017) demonstrated that low (58 mg/day), moderate (143 mg/day) and high (351 mg/day) consumers significantly improved their 30 min cycling time trial performance after 6 mg/kg of acute CAF ingestion. Although sub-chronic (5 days) intake of low (3 mg/kg) or moderate (6 mg/kg) doses of CAF was reported to no moderating effect on thermoregulatory or cardiovascular responses to exercise (Roti et al., 2006), there is no study to date that investigate the tolerance effect on sprint performance. The aim of the current study was to examine, for the first time, effects of low and moderate doses of caffeinated coffee consumption for 1 week on sprint performance responses to acute caffeinated coffee intake.

## 2. Materials and Methods

**Participants** — Twenty-four healthy, non-smoking male team sport players (football, basketball and handball) participated voluntarily in this study (age = 22.0  $\pm$  1.5 years; height = 174.9  $\pm$  7.8 m; body weight = 71.6  $\pm$  11.1; training 5  $\pm$  1 years and 4  $\pm$  1 days in a week). All participant were naïve to the

caffeine ( $23 \pm 1$  mg/day) according to the classification proposed by Filip et al., (2020). Daily caffeine intake level of participants were assessed via a questionnaire (Bühler et al., 2014) also used by previous researches (Gonçalves et al., 2017; Filip-Stachnik et al., 2021). Having sprint type exercise in their training routines, free from musculoskeletal disorders and being naïve caffeine user were followed as an inclusion criteria. Before test sessions, informed consent was signed by all subjects following the Declaration of Helsinki. Ankara University Non-interventional Clinical Research Ethics Committee approved the study procedures (16-1014-17).

**Experimental design** – A double-blind, placebo-controlled and randomized experimental design was used in this study. The coordinating of study procedures was conducted by a researcher who was not involved in data collection and analyses. Each participant visited faculty of sport science's performance laboratory on four separate occasions. The first visit as a familiarization included preliminary testing with all procedures to minimize any learning effects. Next two test sessions were performed with 60 min after ingestion of decaffeinated coffee (PLA) or 6 mg/kg of caffeine provided by coffee (FIRSTCAF) to test acute effect of caffeine on ISP separated by at least 2 days. Following to second test session, participants were randomly divided into three groups (8 athletes for each): 1 week of decaffeinated coffee consumption as placebo (PLAGROUP), 3 mg/kg of caffeine from caffeinated coffee (LOWGROUP) and 6 mg/kg of caffeine from caffeinated coffee (MODGROUP). On the 8<sup>th</sup> day, participants again took 6 mg/kg of caffeine provided by coffee (SECONDCAF) than performed ISP test protocol to test if habituation develops. Body composition (Jawon Segmental Avis 333 Plus, Korea), habituation level of participants and ISP were measured in familiarization session. Participants came to the laboratory after 10 hour night fasting on the morning hours (08.00-10.00 a.m.). Heart rate (HR) (Polar

Team 2 telemetric system, Finland), capillary glucose (GLU) and lactate (LA) (Accutrend Plus, Roche Diagnostics, Germany) from fingertip and ratings of perceived exertion (Borg, 6-20) (RPE) were measured immediately pre, middle and post of ISP test. 12x4 seconds all-out sprint with 90 seconds active recovery at 600 watt were used to test ISP in cycle ergometer (Monark 894 E, Vansbro, Sweden) to simulate team-sports sprint pattern (Lee et al., 2012). To control current research tightly, participants were provided with a list of caffeine containing product and instructed to abstain from heavy physical activity for the last 48 hours, alcohol and caffeine-containing substances within the last 24 hour before test sessions. To standardize macro nutrient intake before 24 hours to experiment, food intake was recorded and replicated before each trial. All study design and test protocols summarized in Figure 1.

#### **Methodology** –

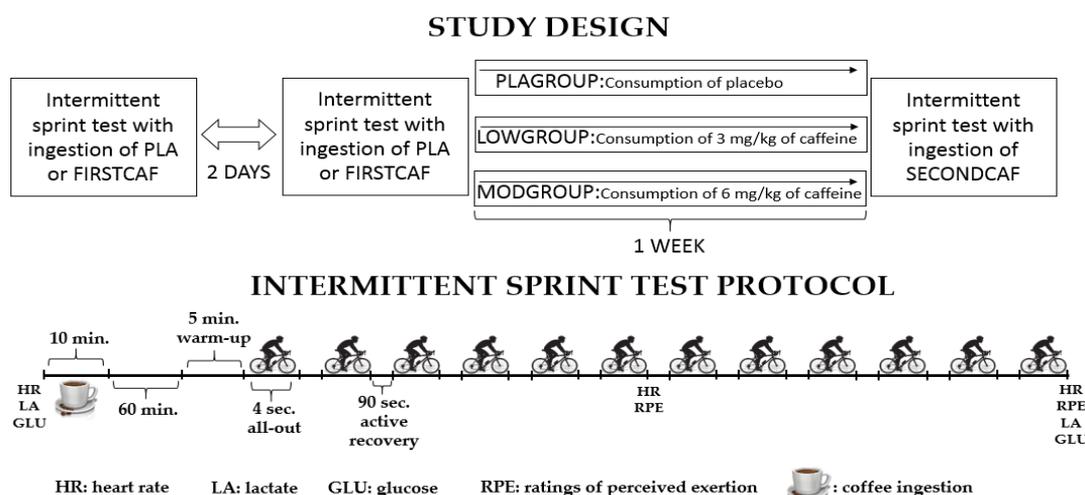
**Intermittent sprint performance test (ISP)** – Participants completed twelve 4 seconds all-out sprints separated by 90 seconds active recovery at 60 Watt (60 rpm against to 1kg) on a cycle ergometer. Seat and handle positions were adjusted to each participants in the familiarization session and this was recorded and remained consistent for all test sessions. Participants were required to stay seated on the cycle ergometer during the whole ISP protocol to limit the recruitment of other muscle groups. The ISP test protocol was designed to replicate team-sport based exercise pattern (Schneiker et al., 2006). Following to 5-min warming-up at 60 Watt, participants cycled maximally during unloaded pedalling and when reached  $\geq 150$  rpm, the test was automatically initiated by Monark test software (Version 3.3.0.0, Vansbro, Sweden) and 4 seconds sprint began with subsequent instant application of load corresponding to 7.5% of body weight. Participants were given strong encouragement to cycle maximally for each 4-s sprints and pedal as fast as possible against the given load but no information feedback. This pattern was replicated 12

times in total. Peak power (PP) and mean power (MP) for each sprint were calculated by Monark Anaerobic Test Software. Capillary GLU-LA, HR and RPE were measured immediately before, after 6<sup>th</sup> sprint and after test.

*Acute or 1 week of coffee consumption protocol* – Acute decaffeinated coffee (PLA) or 6 mg/kg of caffeine provided from coffee (FIRSTCAF) ingestion was performed in the first 2 test sessions 60 min before the beginning of ISP protocol to test acute effects on performance. Following to second test session, all daily doses of coffee measured for each participants of consumption groups were given in a pack at the beginning of 1 week consumption period and asked to ingest with 600 ml of hot water (approximately 60 °C) on the morning hours. It was suggested to participants to consume coffee in 10 min. Instant coffee (Nescafé Gold, Nestlé, Turkey) with decaffeinated and caffeinated versions were prepared for each participant according to their body mass (bm) and consumption group. Recent study stated that 100 gr of Nescafé Gold contains 36 mg of caffeine and decaffeinated version provides very low dose of caffeine to be ergogenic (Karayigit et al., 2020). In this regards, during 1 week of consumption period, PLAGROUP ingested 0.16 gr/kg/bm decaffeinated coffee, LOWGROUP 0.08 gr/kg/bm caffeinated coffee + 0.08 gr/kg/bm decaffeinated coffee (0.16 gr/kg/bm in total) and MODGROUP 0.16 caffeinated coffee in a day. To

standardize the volume of coffee ingested, all groups consumed equal volume of coffee (0.16 gr/kg/bm) to exclude other compounds of coffee that may affect habituation. Further, during 1 week of coffee ingestion, no extra caffeine usage was allowed to all participants and remembered on a daily basis via mobile phone message and social media. Immediately after 1 week of coffee consumption, on the 8<sup>th</sup> day, all participants ingested 6 mg/kg of caffeinated coffee (SECONDCAF) before 60 min to beginning ISP protocol to test whether the habituation to acute caffeine responses occur.

**Statistical analysis** – Data are reported as the mean  $\pm$  the standard deviation (SD). IBM SPSS statistics for Windows, version 22.0 (IBM Corp., Armonk, NY) was used to analyse all data. All variables were analysed with a two-way repeated measured ANOVA. Sphericity was analysed by Mauchly's test of sphericity followed by the Greenhouse-Geisser adjustment where required. One-way repeated measures ANOVA was used to compare the inter-intervention comparisons. If any significant differences were identified, pairwise comparisons with Bonferroni correction were used in order to show where they lay. The statistical significance was set at  $p < .05$ . The effect sized were calculated 95% confidence intervals (CI) and using partial eta squared ( $\eta^2$ ), defined as trivial ( $< 0.10$ ), moderate (0.25-0.39), or large ( $\geq 0.40$ ) (Cohen, 1992



**Figure 1.** The summary of the experimental protocol.

### 3. Results

Although average peak ( $p=0.39$ ;  $\eta^2=0.13$ ) and average mean ( $p=0.11$ ;  $\eta^2=0.15$ ) power of total 12 sprints during ISP test were not statistically different between groups and conditions as seen in figure 2, FIRSTCAF and SECONDCAF significantly increased peak power in the first three sprints compared to PLA ( $p=0.01$ ;  $\eta^2=0.44$ ) in all consumption groups meaning no tolerance was developed in 1 week of consumption 3 or 6 mg/kg/day of caffeinated coffee (figure 3). Post-hoc analysis revealed that FIRSTCAF ( $p=0.01$ ; 95%CI=15.01-91.13;  $p=0.02$ ; 95%CI=5.93-74.90) in first and third sprint and SECONDCAF ( $p=0.01$ ; 95%CI=27.72-83.15;  $p=0.01$ ; 95%CI=16.64-100.41;  $p=0.01$ ; 95%CI=34.34-127.11) in the first, second and third sprint significantly increased peak power compared to PLA, respectively in PLAGROUP. Further, in LOWGROUP, FIRSTCAF ( $p=0.01$ ; 95%CI=24.69-88.94) and SECONDCAF ( $p=0.01$ ; 95%CI=25.88-100.98) significantly enhanced peak power in the first sprint compared to PLA. In MODGROUP, peak power was significantly increased with FIRSTCAF ( $p=0.01$ ; 95%CI=27.30-100.23;  $p=0.01$ ; 95%CI=33.95-92.36) in the first and second sprint and with SECONDCAF ( $p=0.01$ ; 95%CI=4.52-111.33;  $p=0.03$ ; 95%CI=7.05-123.46;  $p=0.01$ ; 95%CI=24.12-150.39) in the first, second and third sprint compared to PLA (Figure 3). Because in all consumption groups, caffeine ingestion significantly increased peak power in the first three sprints, there is no main effect of consumption groups ( $p=0.74$ ;  $\eta^2=0.04$ ) (Figure 3). Similar to peak power, FIRSTCAF and SECONDCAF significantly increased mean power in the first three sprints compared to PLA ( $p=0.01$ ;  $\eta^2=0.46$ ) in all consumption groups. Post-hoc analysis revealed that FIRSTCAF ( $p=0.01$ ; 95%CI=29.76-86.11;  $p=0.03$ ; 95%CI=2.14-64.64) and SECONDCAF ( $p=0.01$ ; 95%CI=35.86-79.56;  $p=0.01$ ; 95%CI=36.48-99.23) significantly increased mean power in the first and third sprints compared to PLA

in PLAGROUP, respectively (figure 4). In LOWGROUP, FIRSTCAF ( $p=0.03$ ; 95%CI=4.98-71.63) significantly increased mean power compared to PLA, in the first sprint. In MODGROUP, FIRSTCAF ( $p=0.01$ ; 95%CI=12.31-101.36;  $p=0.01$ ; 95%CI=19.19-96.46) in the first and second sprints and SECONDCAF ( $p=0.03$ ; 95%CI=2.93-85.86;  $p=0.02$ ; 95%CI=13.68-128.26) in the first and third sprints significantly improved mean power compared to PLA. There is also no main effect of group differences ( $p=0.73$ ;  $\eta^2=0.04$ ) (Figure 4). As expected, from pre to post test, RPE values significantly increased in all groups with ingestion of FIRSTCAF, SECONDCAF or PLA ( $p=0.01$ ;  $\eta^2=0.98$ ). However, there is no effect of group ( $p=0.35$ ;  $\eta^2=0.13$ ), condition ( $p=0.18$ ;  $\eta^2=0.21$ ) and group  $\times$  condition interaction ( $p=0.11$ ;  $\eta^2=0.22$ ). Similarly, HR significantly increased with time ( $p=0.01$ ;  $\eta^2=0.99$ ). However, no significant main effect for group ( $p=0.63$ ;  $\eta^2=0.06$ ), condition ( $p=0.06$ ;  $\eta^2=0.32$ ) and group  $\times$  condition interaction ( $p=0.14$ ;  $\eta^2=0.20$ ) was observed. GLU values also significantly increased with time ( $p=0.01$ ;  $\eta^2=0.88$ ). No main effect for group ( $p=0.60$ ;  $\eta^2=0.07$ ), condition ( $p=0.69$ ;  $\eta^2=0.05$ ) and group  $\times$  condition interaction ( $p=0.32$ ;  $\eta^2=0.14$ ) was detected.

Although LA was not different between groups ( $p=0.63$ ;  $\eta^2=0.20$ ), LA significantly increased in the post-test in all groups ( $p=0.01$ ;  $\eta^2=0.58$ ). Post hoc analysis showed that FIRSTCAF ( $p=0.02$ ; 95%CI=0.23-3.06;  $p=0.02$ ; 95%CI=0.29-3.77;  $p=0.01$ ; 95%CI=1.02-3.45) significantly has high values compared to PLA in PLAGROUP, LOWGROUP and MODGROUP, respectively. However, these significant differences were not observed after 1 week of coffee consumption with SECONDCAF ( $p=0.11$ ; 95%CI=-0.43-3.33;  $p=0.12$ ; 95%CI=-0.51-3.31;  $p=0.86$ ; 95%CI=-1.90-1.63) compared to PLA in PLAGROUP, LOWGROUP and MODGROUP, respectively (Table 1).

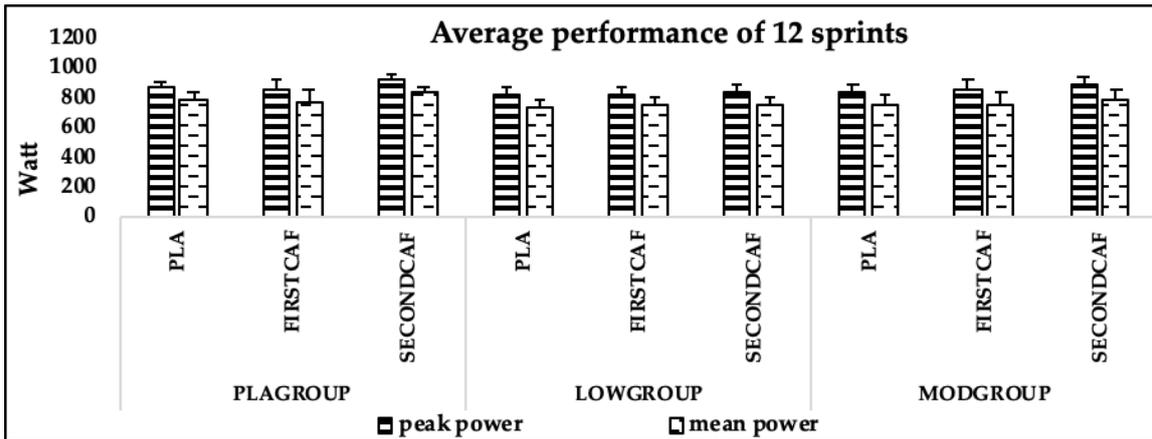


Figure 2. Average values of peak and mean power of all 12 sprints

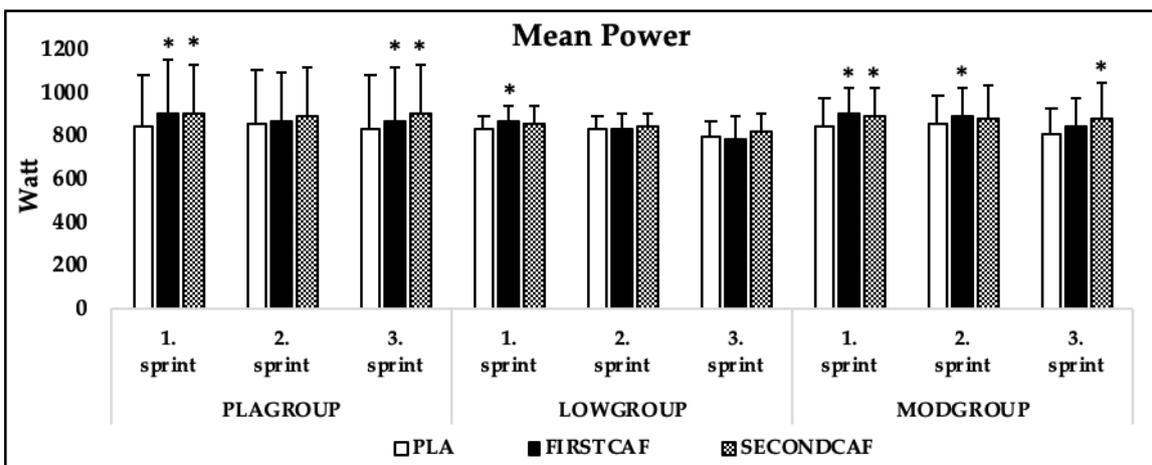


Figure 3. Peak power values of first three sprints. \*: Significantly different from PLA

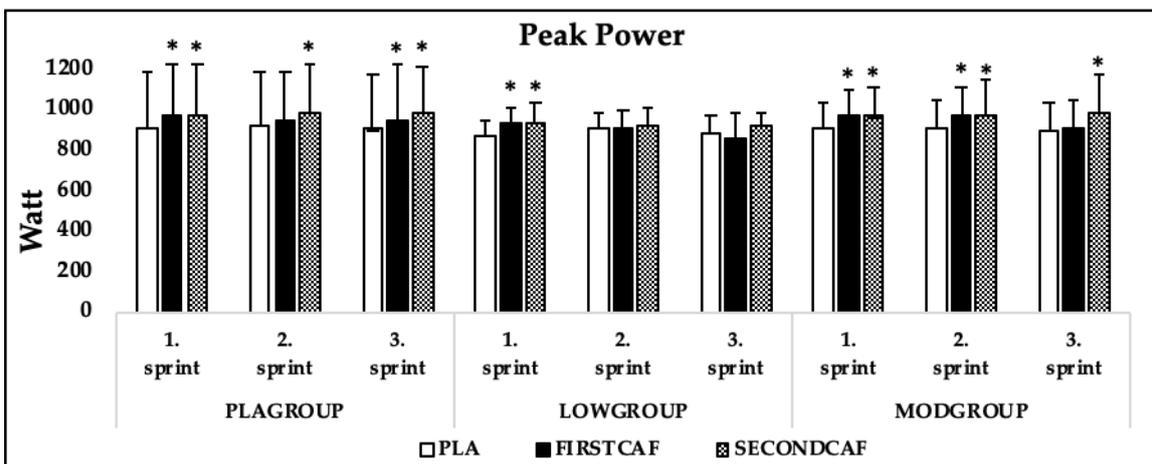


Figure 4. Mean power values of first three sprints. \*: Significantly different from PLA

#### 4. Discussion

This study investigated the effect of 1 week low (3 mg/kg of caffeine) and moderate (6 mg/kg of caffeine) doses of caffeinated coffee consumption on sprint performance responses to acute caffeine ingestion. Novel

design of the current research allowed for an investigation of tolerance effect of 2 different doses caffeine consumption on acute responses. Results showed that acute ingestion of 6 mg/kg of CAF provided by coffee significantly enhanced sprint

performance compared to decaffeinated coffee and 1 week consumption of 3 or 6 mg/kg of CAF provided by coffee does not negatively affect these sprint responses to acute ingestion. Further, there is no acute or 1 week of CAF consumption effect on HR, RPE and GLU. However, acute CAF

ingestion significantly increased LA values at the post-test compared to PLA but this difference was not apparent after consumption of 1 week placebo, 3 or 6 mg/kg of CAF

**Table 1.** Various parameters at different points.

		PLAGROUP			LOWGROUP			MODGROUP		
		PLA	FIRST CAF	SECOND CAF	PLA	FIRST CAF	SECOND CAF	PLA	FIRST CAF	SECOND CAF
<b>GLU (mg/dL)</b>	Pre test	85.6±7.8	85.3±6.6	85.5±6.3	85.2±8.2	87.1±6.5	88.0±7.1	85.5±7.3	83.3±7.9	86.0±6.4
	Post test	101.5±10.4	102.0±13.7	98.6±9.5	103.0±9.1	103.1±7.6	103.2±8.7	98.8±9.0	97.1±5.6	101.2±9.4
<b>LA (mmol/L)</b>	Pre test	1.1±0.3	1.2±0.3	1.0±0.9	1.0±0.3	1.1±0.2	1.1±0.2	1.1±0.2	1.2±0.3	1.1±0.2
	Post test	10.4±2.1	12.1±1.7	11.9±2.5	11.3±3.3	13.0±2.7	11.6±1.8	12.6±1.6	14.8±1.1	12.5±2.5
<b>RPE (6-20)</b>	6. sprint	14.0±1.6	13.6±2.2	13.5±1.6	14.0±1.3	15.1±0.9	14.2±1.0	13.0±1.6	13.7±1.9	14.6±1.5
	12. sprint	17.7±0.8	17.2±2.2	17.3±1.4	17.8±0.9	18.7±1.0	18.5±1.4	17.6±1.1	17.8±1.5	18.7±1.3
<b>HR (beat/min)</b>	Pre test	66.6±6.9	70.6±5.7	70.1±10.5	66.3±7.7	67.0±6.4	69.7±5.9	69.8±9.4	67.5±5.5	68.1±4.1
	6. sprint	164.7±16.0	167.6±12.3	166.8±13.5	163.0±9.6	165.7±7.9	161.7±11.6	164.6±12.2	170.0±13.6	162.3±12.3
	12. sprint	168.7±13.9	173.0±10.0	170.8±12.2	167.2±8.6	165.8±8.5	161.8±12.1	167.5±11.1	169.5±11.5	162.0±11.8

One of two main findings of current study is FIRSTCAF significantly improved sprint performance prior to 1 week of consumption period. Although anhydrous form of CAF was shown to be ergogenic on sprint performance by numerous studies (Dominguez et al., 2021; Glaister et al., 2019; Mohr et al., 2011), current study is first to found 6 mg/kg caffeinated coffee enhanced sprint performance compared to decaffeinated coffee as a placebo. Due to the methodological difficulties, coffee could not

compared with anhydrous form but it is known that both coffee and anhydrous form improves resistance (Richardson et al., 2016) and endurance (Hodgson et al., 2013) exercise performance. Trexler et al., (2016) examined coffee and anhydrous caffeine on sprint performance in low habitual males and reported, despite no statistical difference, coffee may diminish reductions of power output in the later sprints. Due to the distinct coffee taste in Trexler et al., (2016) study, participants were partially blinded and expectancy phenomenon may

come forward. In the current study, it was also not asked to participants to guess what treatment they had ingested. Parallely, coffee was shown to significantly increase sprint cycling performance in those who believed they received caffeine compared to who believed they had received placebo (Anderson et al., 2020). Further, Clarke et al., (2016) investigated if effects differ between coffee and anhydrous form of 3 mg/kg of CAF on ISP and reported no benefit of both anhydrous and coffee form in untrained males. Low dose of CAF intake may affect the results of Clarke et al., (2016) study because most of the studies in the literature found improvement in sprint performance with 5-6 mg/kg of CAF ingestion (Grgic, 2018). Turley et al., (2015) was also reported to 3 and 5 mg/kg of caffeine intake may enhance peak and mean power divergently. Conversely, well-designed research by Glaister et al., (2012) demonstrated none of doses between 2-10 mg/kg of anhydrous CAF improved short-duration sprint cycling performance. Surprisingly, 3 mg/kg of CAF was found to be beneficial on peak and mean power output but same effect was not observed with 6 and 9 mg/kg doses (Wang et al., 2020). A few test variable can be speculated to explain various reports. Training status of participants may moderate ergogenic magnitude of acute CAF intake because concentration of adenosine receptors (main mechanism of CAF) is higher in trained athletes compared to untrained (Clarke et al., 2016). Current study's participants were well-trained team-sport players and respond to acute and sub-chronic CAF intake. Although CAF's ability to improve muscular endurance performance was found to be no different between trained and untrained individuals by a meta-analysis (Warren et al., 2010), more research is needed to investigate training status's effect on sprint performance responses to acute CAF intake. Further, acute CAF increase sprint performance commonly in low but not high habitual participants (Evans et al., 2018). In the current study, participants were very low habitual users ( $\leq 25$  mg/day) and

responded to 6 mg/kg of CAF. However, 200 mg of acute CAF intake was reported by Evans et al., (2018) to improve repeated sprint performance in team sport athletes with low, but not with moderate-to-high habitual users. Lastly, rest interval duration between sprints may also affect responses to acute CAF which showed by Lee et al., (2012) that 5 mg/kg of anhydrous CAF ingestion improved 12x4 seconds intermittent sprint cycling performance with 90 seconds recovery (as in the current study) but not with 20 seconds recovery.

Repetitive consumption of CAF has been associated with an upregulation of the number and activity of adenosine receptors found in the vascular and neural tissues of the brain (Beaumont et al., 2017). Differences in performance response to acute CAF intake between low and high habitual users is not consistent and study results remain equivocal. In one study, 4 weeks of 3 mg/kg of CAF consumption resulted in ergogenic effect of acute CAF intake no longer apparent on aerobic endurance performance in low habitual users (Beaumont et al., 2017). This results are in contrast with our study that found both 3 and 6 mg/kg of CAF intake for 1 week did not develop further tolerance to acute 6 mg/kg of CAF on sprint performance. Differences between studies are consumption duration, form of CAF and test variable (aerobic endurance vs sprint). It can be speculated that antioxidant components found in coffee may modify tolerance development which can explain different results between Beaumont et al., (2017) and current study. Duration of consumption period (1 week vs. 4 week) may be another confounding factor between studies, however, supporting to our results, 6 mg/kg of CAF intake for 4 days does not induced tolerance to the ergogenic effects promoted by acute intake on 16 km time-trial performance even in already moderate to high (285 mg/day) consumers (Morales et al., 2020). It was also reported by two studies that 3 mg/kg of CAF intake chronically for 20 days enhanced aerobic exercise of submaximal intensity and peak cycling power during wingate test in low habitual

users (Ruiz-Moreno et al., 2020; Lara et al., 2019). However, although CAF was still ergogenic after this period of time, both studies declared a progressive attenuation of the size of the ergogenic effect. Further, Gonçalves et al., (2017) showed 30 min cycling time trial performance effects of acute 6 mg/kg of CAF intake were not influenced by the level of habitual consumption. Important limitation of Gonçalves et al., (2017) study was 24 hour CAF withdrawal period of which may affect performance results particularly in high consumers by reversing CAF withdrawal effects rather than acute CAF ingestion. In our study, withdrawal effect is not relevant because participants were already very low habitual consumers (< 25 mg/day). On the other hand, Irwin et al., (2011) demonstrated that acute 6 mg/kg CAF intake significantly enhances high-intensity endurance cycling performance, regardless of whether a period of withdrawal or consumption (3 mg/kg of CAF) for 4 days in habitual users. Our and most of the previous studies (Irwin et al., 2011; Gonçalves et al., 2017; Morales et al., 2020) used acute CAF doses exceeded the average amount of usual consumption that may favour the absence of tolerance development. Conversely, when acute CAF intake (3 mg/kg) was equal to which consumed (3mg/kg) for 4 weeks, tolerance was then reported to developed (Beaumont et al., 2017). Further research is required to test if tolerance develops when acute ingestion of CAF ( $\leq 3$  mg/kg) is lower than consumed ( $\geq 6$  mg/kg) for a longer time period (4-8 weeks).

Current results showed 6 mg/kg of caffeinated coffee intake does not change heart rate, capillary glucose and RPE values. This findings are in line with some studies (Glaister et al., 2018; Clarke et al., 2016; Schneiker et al., 2006; Clarke et al., 2018) that showed, commonly, caffeine has no significant effect on these variables. There are also reports to demonstrated higher heart rate (Turley et al., 2015) and lower RPE (Doherty et al., 2004; McLellan et al., 2004) values with CAF ingestion. Further,

although acute 6 mg/kg of caffeinated coffee intake significantly increased post-test lactate levels, same effect was not apparent after 1 week of coffee consumption period even in placebo group. CAF may increases blood lactate levels by stimulating glucose uptake in skeletal muscles during intermittent sprint with short rest interval (Lee et al., 2012). Graham et al., (1998) also suggested that CAF may inhibit blood lactate clearance rather than production. However, in our study, it seems that 1 week of coffee consumption blunt CAF's moderating effect on blood lactate levels. Antioxidant and anti-inflammatory properties of coffee appeared to alter the metabolic effects but not the ergogenic effects on performance (Hodgson et al., 2013).

The current study has a few limitations that should be addressed. First, the study included 24 participants, though it was comparable to studies in this topic (Beaumont et al., 2017; Filip-Stachnik et al., 2021). Although, 12x4 seconds intermittent sprint cycling test with 90 seconds active recovery is a valid laboratory method (Lee et al., 2012; Girard, 2011), these findings should be confirmed in running based field tests. Additionally, a post-1 week consumption placebo trial would have been added to directly compare with SECONDCAF trial which may direct participants "expectancy" effect. Participants' habitual CAF intake in the current study was very low and 1 week consumption period might be short to influence the number and activity of adenosine receptors. Future research should investigate various doses of CAF consumption for longer term in moderate to high consumers. Lastly, due to blood concentration of some neurotransmitters were not measured as an indicators of central noradrenergic and dopaminergic activity, exact mechanisms are not know by which 6 mg/kg of caffeinated coffee significantly increased sprint performance.

## 5. Conclusions

In summary, the current study concludes that acute ingestion of 6 mg/kg of caffeine from coffee significantly increase mean and peak power output, and this performance benefits did not disappear after 1 week of low (3 mg/kg of caffeine) or moderate (6 mg/kg of caffeine) doses of caffeinated coffee consumption. Therefore, team sport players with low habitual caffeine usage may wish to plan their micro cycle supplement strategy to increase their sprint performance by ingesting moderate dose of caffeinated coffee even after 1 week of same dose of caffeine consumption.

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