Official Title: Varying Doses of Evening Caffeine Ingestion Have Different Effects on Rowing Ergometer Performance, Sleep Quality and Wakefulness Scores

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Varying Doses of Evening Caffeine Ingestion Have Different Effects on Rowing Ergometer Performance, Sleep Quality and Wakefulness Scores

Abstract

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3 This study investigated the dose-dependent effects of evening caffeine ingestion on rowing 4 performance, sleep quality, and daytime sleepiness in trained male rowers. Employing a double-blind, randomized, crossover design, 13 university-level rowers completed four 2000-meter time trials under 5 6 placebo (PLA), low (3 mg/kg, LDC), moderate (6 mg/kg, MDC), and high (9 mg/kg, HDC) caffeine conditions. Performance metrics, heart rate, and subjective sleep assessments were collected. Results 7 8 indicated significantly improved rowing times and power output with HDC and MDC compared to 9 PLA (p < 0.05), with HDC yielding the most notable enhancements (d = 0.40-0.41). However, these 10 ergogenic benefits were accompanied by significantly impaired sleep quality and elevated daytime sleepiness in both HDC and MDC groups (p < 0.01, d = 1.3-1.5). Notably, adverse effects such as 11 headache, insomnia, and gastrointestinal discomfort were predominantly reported in the HDC 12 condition (p < 0.05). Although LDC offered mild performance improvements with minimal sleep 13 14 disruption, only the high dose condition exhibited large physiological and perceptual trade-offs. These findings indicate a clear dose–response relationship, wherein higher evening caffeine intake improves 15 16 performance but have detrimental effects on sleep and recovery markers. Coaches and athletes should carefully balance caffeine dosing against potential recovery costs, especially in evening training or 17 competition contexts. 18

1 Introduction

- The use of caffeine, a component of the methylxanthine group, as an ergogenic aid is widely preferred by athletes to increase physical and cognitive performance (Pickering & Grgic, 2020). More than 75% of athletes in various branches take caffeine supplements to gain an advantage before or during competition (Del Coso et al., 2011; Van Thuyne et al., 2005). Caffeine use has been reported to increase, especially in individual and aerobic-based branches. Additionally, among the analyzed athletes, rowers appear to be among the highest caffeine users (Aguilar-Navarro et al., 2019).
- 26 The desired form of caffeine can be chosen, including coffee, capsule, gum, bar, gel and aerosol 27 (Wickham & Spriet, 2018). Acute caffeine intake has been confirmed to have a positive effect on 28 athletic performance related to parameters such as cardiovascular endurance (Southward et al., 2018), 29 anaerobic endurance (Grgic, 2018), movement speed (Raya-Gonzalez et al., 2020), power (Grgic et al., 2018) and muscular endurance during resistance exercises (Polito et al., 2016). The most widely 30 known mechanism of caffeine's ergogenic effects is through antagonism of adenosine receptors, which 31 in turn reduces the concentration of several central nervous system neurotransmitters, including 32 33 serotonin, dopamine, acetylcholine, norepinephrine, and glutamate (Fredholm, 1995). Caffeine 34 compounds inhibit the known effect of adenosine by binding to adenosine receptors due to their similar structure to adenosine. It is known to improve aerobic-muscle endurance performance by increasing 35 the release of neurotransmitters that promote wakefulness (Astorino & Roberson, 2010; Guest et al., 36
- 37 2018; Higgins et al., 2016; Mohr et al., 2011).

Rowing is a sport that is likely to be affected by natural circadian (24-hour) or diurnal (time of day) changes because significant muscle strength is required especially in the drive phase of the rowing stroke(Steinacker, 1993). It has been thought by researchers that the ergogenic effects of caffeine can be used to compensate for possible decreases in performance. However, despite its potential to improve performance, caffeine can cause side effects such as headache, nausea, insomnia or anxiety (Wikoff et al., 2017). In a meta-analysis examining the side effects that occur in athletes after caffeine supplementation, it was reported that there was a 34% higher probability of side effects after consuming low and moderate doses of caffeine (de Souza et al., 2022). The same meta-analysis study emphasized that heart palpitations and sleep problems were the most frequently reported side effects. Considering the negative effects of sleep deprivation on exercise performance (Bonnar et al., 2018; Fullagar et al., 2015), it is important to identify the conditions that pose a risk to athletes' sleep quality. The effects of afternoon caffeine intake on sleep efficiency the following night have been the focus of some researchers. Moderate doses (6 mg/kg) of caffeine taken before cycling exercises at approximately 5:00 PM delayed falling asleep and shortened total sleep time (Miller et al., 2014). Another similar study reported that 6 mg/kg of caffeine taken before an 800-meter running test at 8:00 PM reduced sleep efficiency (Ramos-Campo et al., 2019). In the same study negative feedback was received from athletes for subjective sleep parameters such as "sleep quality," "calm sleep," "ease of falling asleep," and "feeling refreshed after waking". Other studies involving rugby players have also found negative or neutral findings on caffeine-related sleep quality (Caia et al., 2021; Dunican et al., 2018) In the study conducted by Caia et al. (2021) post-competition salivary caffeine concentrations of athletes who continued their usual caffeine consumption before or during the competition were analyzed. A moderate negative relationship was found between the increase in caffeine levels and sleep onset delay and sleep efficiency. This effect of caffeine on sleep efficiency is attributed to the time it takes to be metabolized. After caffeine is taken, its concentration in the blood plasma typically reaches its highest level within 60 minutes and it takes approximately 4-6 hours for half of the initial dose to be metabolized (Burke, 2008). Therefore, caffeine consumed before going to bed reduces sleep duration and sleep efficiency (Drake et al., 2013). In addition, it can be said that caffeine reduces sleep efficiency due to the tendency of the individual to temporarily increase the number of awakenings during the sleep period (McHill et al., 2014). Therefore, total sleep time will also decrease.

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There are conflicting studies on the optimum caffeine dose in caffeine consumption (Clarke et al., 2016; Spriet, 2014). In addition, randomized studies in the literature where different doses are observed in the same sample group are limited. Moreover, verification of the effect of different caffeine doses on sleep quality will provide valuable information especially for athletes who use caffeine before evening training or competitions (Burke, 2017). Moderate to high doses (6-9 mg/kg) of caffeine significantly improve short-duration, high-intensity rowing performance by reducing the time required to complete the distance on a 2000 m rowing ergometer (Bruce et al., 2000). Similarly, 6 mg/kg of caffeine is observed to significantly improve mean power output in 2000 m rowing performance (Gharaat et al., 2020). Contrary to these findings, in the study conducted by Skinner et al. (2010), it was concluded that 2, 4 and 6 mg/kg of caffeine did not provide any improvement in performance. The conflicting results regarding caffeine and rowing performance suggest that further randomized studies are needed. Furthermore, the fact that low-dose caffeine supplementation (<3 mg/kg) generally improves athletic performance, as well as the low risk of side effects reported (Spriet, 2014), suggests that the negative effects of caffeine on sleep quality can be minimized. This is supported by a study conducted by Filip-Stachnik et al. (2021) on judokas. After consuming 3 mg/kg of caffeine in the evening (19:00), they concluded that low-dose caffeine did not cause a significant deterioration in objective sleep parameters after the following night's sleep analysis of athletes who trained. However, the lack of studies in the literature that evaluate the effects of caffeine use on performance, as well as the effects on sleep quality the night following use and the level of alertness the next day, is striking.

- 86 In this context, the aim of the present study was to examine the effects of different doses of caffeine
- taken in the evening on (1) ergometric rowing performance, (2) sleep quality, and (3) alertness levels.
- 88 This research raised the following hypotheses: I different doses of caffeine will produce different
- 89 performance and sleep-related responses. II low, medium and high doses of caffeine supplements
- 90 taken in the evening will improve athletes' performance on the rowing ergometer (time trial and average
- 91 power output). III caffeine intake, except for low doses, will negatively affect sleep quality and
- 92 increase sleepiness levels.

2 Material Methods

94 **2.1 Study Design**

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- 95 The research employed a double-blind, crossover design with randomization. Participants engaged in
- 96 2000-meter indoor rowing time trials during four separate laboratory visits. Time trials were conducted
- 97 under four primary testing conditions involving the intake of: 3 mg/kg caffeine, 6 mg/kg caffeine, 9
- 98 mg/kg caffeine or placebo. All testing sessions were carried out in a manner that was both randomized
- 99 and counterbalanced. Participants were randomized by the independent researcher to the intervention
- or placebo groups using online research randomizer (https://www.randomizer.org/) which uses the
- "Math.random" method within the JavaScript programming language to generate its random numbers
- 102 (Urbaniak and Plous, 2013).
- 103 Every time trial was performed using a Concept II Model D Rowing ergometer (Concept II,
- Morrisville, VT, USA), with both the completion time and average power output (measured in watts)
- being documented. Additionally, heart rate measurements were taken during the tests utilizing a Polar
- Team Pro system equipped with an H10 sensor (Polar Electro OY, Kempele, Finland). The testing
- sessions were spaced at least 72 hours apart, with a maximum interval of seven days, to guarantee
- adequate washout of treatments and sufficient recovery time.
- 109 Each participant's warm-up routine was documented during the initial trial and then consistently
- reproduced in the subsequent trials. Testing sessions were uniformly scheduled in the evening, between
- 111 19:00 and 20:00, and at identical times for each participant to mitigate the influence of circadian
- 112 rhythms. Upon the conclusion of each experimental trial, participants were asked to report any side
- effects they might have encountered.

114 2.2 Participants

- The investigation involved a group of 13 male university-level rowers, with an average age of 22.07
- years (standard deviation [SD] = 2.21), body mass of 77.66 kg (SD = 6.45), height of 182.14 cm (SD
- = 7.11), body fat percentage of 11.23% (SD = 4.1), and a typical daily caffeine consumption of 303.62
- mg (SD = 148.34). These individuals had accrued an average of 3.1 years (SD = 1) of rowing training
- 119 experience.
- The required sample size was determined using G*Power software (version 3.1.9.4; Dusseldorf,
- 121 Germany), based on an analysis of variance (ANOVA) design incorporating repeated measures and
- within-subjects factors. The calculation utilized an effect size (f) of 0.25, a significance level (alpha)
- of 0.05, and a statistical power of 0.95 and r=0.85 with a single cohort of participants.
- During their initial laboratory visit, participants underwent anthropometric assessments. Height and
- body mass were recorded using a Seca stadiometer (Seca Deutschland, Hamburg, Germany), while
- body fat percentage was evaluated with an InBody 770 body composition analyzer (InBody Co.,

- 127 Gangnam-Gu, Seoul, Korea). Following these measurements, participants were provided with detailed
- study protocol information sheets and signed informed consent forms. 128
- 129 Eligibility for participation was restricted by specific exclusion criteria. Rowers were excluded from
- 130 the study if they presented with:

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- 131 1. A medical condition that impaired their capacity to follow the study protocol;
- Current use of prescription medications; 132 2.
 - 3. A confirmed allergy to mannitol or any other sweeteners;
- 134 4. A diagnosed sleep disorder; or
- 135 A physician's recommendation to limit or avoid caffeine intake. 5.

Supplementation Protocol

137 Capsules, identical in appearance, were used to deliver the substances for all conditions. The dosages

- of caffeine were individually calculated based on each participant's weight. Placebo capsules were
- 139 filled with sugar alcohol (mannitol), not expected to have any further effects on performance (Flueck
- 140 et al., 2016). Caffeine capsules contained caffeine powder. In both caffeine and placebo experiments,
- 141 participants consumed 3 to 5 gelatin capsules, with the quantity of capsules standardized across all
- 142 subjects. Researchers and participants could not distinguish between placebo and caffeine capsules due
- 143 to equal color and size. At the end of each trial, participants were asked to indicate their assumption
- 144 regarding the type of capsules swallowed. The capsules were provided 60 minutes before each trial in
- 145 order to have sufficient time to ensure increasing blood caffeine levels (Pallares et al., 2013). The
- 146 supplementation groups were as follows; 3 mg/kg caffeine (low dose caffeine, LDC), 6 mg/kg caffeine
- 147 (moderate dose caffeine, MDC), 9 mg/kg caffeine (high dose caffeine, HDC) or placebo (PLA). Prior
- 148 to departing the laboratory, participants were queried regarding their perception of the treatment
- 149 administered, specifically whether they believed they had received no caffeine, a low dose, a moderate
- 150 dose, or a high dose. Furthermore, physical exhaustion and adverse effects (such as gastrointestinal
- 151 issues, tachycardia, muscle pain, or headaches) experienced 24 hours post-supplement administration
- 152 were documented via an online survey, following the methodology described by Pallares et al. (2013).
- 153 Owing to the physically demanding nature of the rowing time trial, the collection of side effect
- 154 responses was scheduled exclusively for 24 hours post-test.

2.4 **Diet and Caffeine Consumption Control**

- 156 The research participants were instructed to abstain from alcohol consumption and vigorous physical
- 157 training for a 24-hour period preceding each experimental session. Throughout the duration of the
- 158 study, they were advised to refrain from using any dietary supplements. All participants were required
- 159 to maintain a detailed 24-hour dietary record on the day prior to each testing session, as well as a
- 160 weekly log of their caffeine intake. To ensure consistency in energy consumption and hydration status,
- 161 participants were directed to replicate their dietary intake, as documented in the food log, before every
- trial. Daily caffeine consumption was quantified using a modified version of the questionnaire 162
- 163 developed by Bühler et al. (2014). Additionally, the caffeine content from various food and beverage
- 164 sources was incorporated to determine the total daily caffeine intake. Based on this evaluation, all 165 participants were classified as habitual moderate caffeine consumers, in accordance with the criteria
- 166 established by Filip et al. (2020). To simulate conditions reflective of real-world athletic environments,
- 167 as recommended by Tallis et al. (2021), participants were encouraged to maintain their usual daily
- 168 caffeine intake throughout the study. This approach was implemented to mitigate the potential impact
- 169 of caffeine withdrawal, as noted by Pickering and Kiely (2019).

Subjective Sleep Quality and Daytime Sleepiness Measurements 2.5

- 171 Participants were directed to maintain their normal sleep patterns both prior to and following the
- 172 experimental sessions. Each morning subsequent to a trial, they assessed their sleep quality utilizing a
- validated numeric rating scale, with scores ranging from 0 (indicating "best possible sleep") to 10 173
- 174 (denoting "worst possible sleep") (Snyder et al., 2018). Furthermore, levels of daytime sleepiness were
- 175 evaluated in the afternoon through the application of the Karolinska Sleepiness Scale (Shahid et al.,
- 176 2012), which employs a numeric range from 1 (representing "extremely alert") to 9 (signifying "very
- 177 sleepy").

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2000-m Rowing Time Trial 2.6

- 179 The 2000 meters time trial was performed on the same rowing ergometer for each participant. The test-
- 180 retest reliability of 2000m time trial on the Concept II rowing ergometer has previously been examined
- 181 previously with well-trained rowers and was reported with a coefficient of variation (CV) of 0.6%
- 182 (Schabort et al., 1999). Each participant's warm-up routine was documented during the initial trial and
- 183 then consistently reproduced in the subsequent trials. The time to complete the time trial was recorded.
- 184 The stroke rate during the test was freely selectable by each subject and the drag factor settings of the
- 185 ergometers were adjusted to 140 as recommended by Amateur Rowing Association for heavyweight
- 186 men rowers (O'Neill & Skelton, 2004). During the 2k test, after a self-selected warmup, the athletes
- 187 were required to row 2000m in the least time possible. This test is a standard criterion used for national
- team selection purposes in many countries (Hahn et al., 2000; Pripstein et al., 1999) and was performed 188
- 189 routinely by all rowers in this study.

2.7 **Statistical Analysis**

- 191 The normality of the dataset was initially assessed using the Shapiro-Wilk test. Following this, the
- 192 sphericity assumption was evaluated with Mauchly's test, and the Greenhouse-Geisser correction was
- 193 implemented whenever violations of sphericity were evident. To examine differences in test
- 194 completion time, heart rate, power output, and subjective sleep and daytime sleepiness parameters
- 195 across the full duration of the testing period, a repeated measures analysis of variance (ANOVA) was
- 196 employed. The partial eta square (np2) was utilized to assess the effect size, which was classified as
- 197 small (0.10–0.24), moderate (0.25–0.39), or large (≥ 0.40). Where significant effects emerged,
- 198 Bonferroni post hoc paired comparisons were conducted to identify specific differences between
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- conditions. Cohen's d effect sizes for repeated measures, reported alongside their 95% confidence
- 200 intervals (95% CI). These effect sizes were interpreted according to the following benchmarks: values
- less than 0.20 were deemed trivial, those ranging from 0.20 to 0.49 were classified as small, 0.50 to 201
- 202 0.79 as moderate, and values of 0.80 or higher as large (Cohen, 1992). Statistical computations were
- 203 executed using SPSS software (version 30; IBM Corp., Armonk, New York, USA), while data
- 204 visualizations were created with GraphPad PRISM Software (Version 10.4, GraphPad Inc., San Diego,
- 205 CA, USA). Additionally, a chi-square analysis was employed to investigate differences in the
- 206 prevalence of side effects associated with varying caffeine doses among athletes 24 hours post-
- 207 administration.

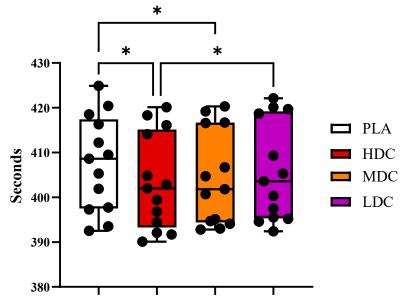
3 **Results**

- 209 Effects of different supplementations on rowing ergometer time trial time have been demonstrated in
- 210 figure 1.

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Figure 1.

2000 m. rowing ergometer Time Trial completion time.



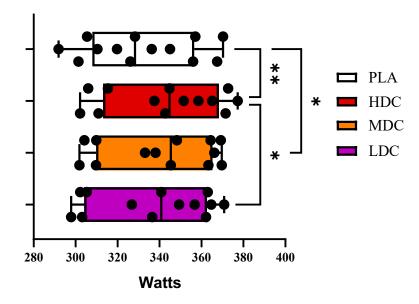
PLA=Placebo, HDC=High Dose Caffeine, MDC=Moderate Dose Caffeine, LDC=Low Dose Caffeine, *=p<0.05

There was a significant difference in rowing time trial performance between conditions (p=0.001, ηp2=0.45). Bonferroni post-hoc analysis showed time trial completed faster in HDC condition (403.30±10.66 seconds) than PLA (407.62±10.77 seconds, p=0.013; 95% Confidence Interval (CI)=0.807–7.793; d=0.40). Time trial performance was significantly better in MDC (404.29±10.57 seconds) group compared to PLA (407.62±10.77, p=0.039; 95% CI=0.136–6.356; d=0.30). In addition, the results were significantly better for HDC group (403.30±10.66 seconds) compared to LDC group (405.68±11.07 seconds, p=0.024; 95% CI=-4.590 – -0.271; d=0.23). Despite non-significance, mean values of LDC (405.68±11.07 seconds, p=0.197; d=0.13) were superior to PLA (407.62±10.77 seconds) which could be practically relevant in the field settings.

Mean power output during Time trial has been provided in Figure 2

Figure 2

Mean power output during 2000 m. rowing ergometer Time Trial.



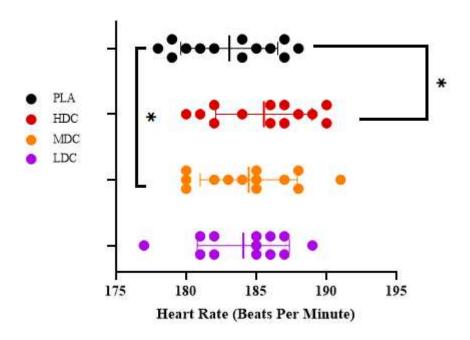
PLA=Placebo, HDC=High Dose Caffeine, MDC=Moderate Dose Caffeine, LDC=Low Dose Caffeine, *=p<0.05, **=p<0.01.

There was a significant difference in time trial mean outputs between conditions (p=0.001, ηp2=0.47). Bonferroni post-hoc analysis showed higher power outputs for HDC (342.77±26.67 watts) compared to PLA (332.1±25.9 watts, p=0.009; 95% CI=-19.118 – -2.513; d=0.41). The results were significantly better for HDC group (342.77±26.67 watts) compared to LDC group (336.88±27.05 watts, p=0.028; 95% CI=0.537–11.263; d=0.23). In addition, higher power outputs for MDC (340.24±26.24 watts) were detected compared to PLA (332.1±25.9 watts, p=0.027; 95% CI=-15.782 – -0.788; d=0.31).

Average heart rate values obtained during time trial have been presented in figure 3.

Figure 3

Average heart rate during 2000 m. rowing ergometer Time Trial.



PLA=Placebo, HDC=High Dose Caffeine, MDC=Moderate Dose Caffeine, LDC=Low Dose Caffeine, *=p<0.05.

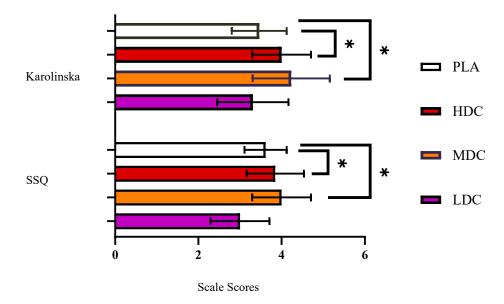
There was a significant difference in heart rate values between conditions (p=0.001, $\eta p2$ =0.56). Bonferroni post-hoc analysis showed higher hear rate for HDC (185.54 \pm 4.43 beats per minute [bpm]) compared to PLA (183.08 \pm 3.88 bpm, p=0.002; 95% CI=-3.228 – -1.695; d=0.72). In addition, the results were significantly different between MDC group (184.46 \pm 4.34 bpm) and PLA group (183.08 \pm 3.88 bpm, p=0.005; 95% CI=-2.365 – -0.405; d=0.40).

Subjective sleep quality and Karolinska daytime sleepiness scale values has been provided in Figure 4.

There was a significant difference in sleep variables between conditions (p=0.002, $\eta p2=0.42$). Bonferroni post-hoc analysis showed worse sleep quality for HDC (4±0.71) compared to PLA (3±0.77, p=0.005; 95% CI=-1.714 – -0.286; d=1.5). Significantly worse sleep quality scores have been reported in MDC (3.85±0.69) trial than PLA (3±0.77, p=0.032; 95% CI=-1.632 – -0.060; d=1.3). On the other hand, participants in LDC trial reported worse sleep quality despite non-significance, values were close to significance (p=0.082) and may have a practical effect during practice. significant difference in sleep variables between conditions (p=0.002, $\eta p2=0.42$).

Significant differences in daytime sleepiness have been reported between conditions (p=0.002, $\eta p2=0.37$). Daytime sleepiness values reported in HDC trial (4.31±0.95) were significantly higher than PLA (3.23±0.73, p=0.009, 95% CI=-1.911 – -0.243; d=1.4). Similarly, daytime sleepiness values reported during MDC trial (4.23±0.73) were significantly higher than PLA (3.23±0.73, p=0.005, 95% CI=-1.714 – -0.286; d=1.3).

Figure 4Subjective sleep quality and daytime sleepiness values.



PLA=Placebo, HDC=High Dose Caffeine, MDC=Moderate Dose Caffeine, LDC=Low Dose Caffeine, *=p<0.05.

Reported side effects after 24 hours post-test was presented in Table 1.

Table 1. The incidence of side effects reported by participants (n = 13)

| Variables | PLA | LDC | MDC | HDC |
|----------------------------|-----|-----|-----|--------------|
| Increased urine output | 0 | 1 | 2 | 5*# |
| Muscle soreness | 1 | 1 | 2 | 2 |
| Headache | 0 | 0 | 1 | 5*#† |
| Tachycardia | 0 | 0 | 2 | 4*# |
| Increased vigor/activeness | 0 | 0 | 1 | 2*# |
| Anxiety or nervousness | 0 | 0 | 1 | 4*# † |
| Gastrointestinal problems | 0 | 0 | 1 | 6*#† |
| Insomnia | 0 | 2 | 5 | 7*# |

PLA=Placebo, HDC=High Dose Caffeine, MDC=Moderate Dose Caffeine, LDC=Low Dose Caffeine,

*=p<0.05 vs. PLA, #=p<0.05 vs. LDC, †=p<0.05 vs. MDC.

Significantly higher prevalence of adverse side effects such as headache, gastrointestinal problems, increased urine output and insomnia were reported after HDC condition compared to all other conditions. Just three participants accurately discerned two out of the four experimental conditions, while another three correctly identified only one condition. Collectively, these results suggest that the randomization process was effective. Only one participant out of thirteen accurately identified the full set of experimental conditions, indicating that the blinding procedure was successful.

4 Discussion

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The current study presents an experimental design that examines the effects of different doses of caffeine (low: LDC, medium: MDC, high: HDC) taken in the evening on timed rowing performance, cardiovascular responses, sleep quality, and daytime alertness. The main findings of the study showed that MDC and HDC in particular produced ergogenic effects in terms of performance, but these effects were accompanied by significant sleep disruptions and side effects with increasing doses.

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When previous studies were examined, it was reported that they performed better in the afternoon (17:00) compared to the morning session in the 5-meter multiple shuttles run test (Souissi et al., 2019). In another study, when the effect of time of day on repeated sprint performance was examined, higher muscle strength was reported during the afternoon hours or early evening compared to the morning hours (Aloui et al., 2013). However, these studies did not evaluate the athletes' fixed-distance time trial performances. On the other hand, in a study conducted specifically on rowers, in which performances on the 2000m rowing ergometer in the morning and evening were evaluated, it was reported that they performed an average of 2.4 seconds faster in the morning training compared to the evening, regardless of chronotype (Brown et al., 2008). Considering this research, it is understandable that rowers need supplements to optimize their performance in the evening training. In the current study where the test sessions were conducted in the evening hours, the 2000 m rowing ergometer completion time was significantly shorter in the HDC (9 mg/kg) and MDC (6 mg/kg) conditions compared to PLA, indicating a dose-dependent performance increase. Although statistical significance was not obtained in the LDC (3 mg/kg) condition, the decrease in mean time may be important in practical applications. Even 1% changes in mean speeds in intense Olympic endurance events lasting approximately 45 seconds to 8 minutes are sufficient to affect the medal rankings of athletes (Christensen et al., 2017). Especially in rowing, which is a race against time, gains of seconds may be decisive. The findings of the current study are consistent with previous studies on the effects of caffeine on short-term highintensity performance (Anderson et al., 2000; Bruce et al., 2000; Davis & Green, 2009; Southward et al., 2018). Similar to our findings, Bruce et al. (2000) in an experimental study in which diet and training were well controlled, it was observed that caffeine (6 mg/kg or 9 mg/kg) consumed before a short-term high-intensity endurance test provided a significant increase in performance. In another study, 3 mg/kg caffeine intake in the evening did not significantly improve athletes' 100m swimming time trial performances (Newbury et al., 2022). Doherty et al. (2004) reported that caffeine had a more limited ergogenic effect in intense and short-term exercises compared to long-term endurance exercises. Accordingly, it can be thought that higher doses are required for the ergogenic effect of caffeine in short-distance time trial performances to be seen. In addition, the average daily caffeine consumption of the participants in the current study was 303.62 mg (SD = 148.34). Considering the possibility of developing tolerance to caffeine, the amount consumed by the participants in the lowdose caffeine condition is quite low compared to their daily habits. This may explain why the improvement in time trial performance observed in the high and medium dose caffeine conditions was more pronounced compared to the low dose condition.

Power output is usually the primary performance measure for ergometer tests. It is essential to establish a reliable rowing performance test to test the effectiveness of an intervention on a rower's power generation ability (Soper & Hume, 2004). The mean percentage standard error (%SEM) for mean power between repeated 2000 m performances on the Concept II ergometer has been reported as 2.0% (Schabort et al., 1999). In other words, it can be said that the 2.0% difference in mean power in repeated tests is due to natural measurement variability. In the present study, the 3.2% increase in mean power obtained in HDC and PLA conditions is above the 2.0% natural measurement variability. This supports the possibility that the observed difference is due to an ergogenic effect. There is also a significant difference between MDC and PLA. MDC supplementation resulted in a 2.45% increase in average strength compared to PLA. Similar to the HDC condition, this increase over the natural measurement

variability supports the significant effect of the MDC intervention. The findings of the present study are consistent with the literature. Chen et al. (2024) in their meta-analysis on time trial performance of cyclists, supported that moderate dose caffeine intake led to significant improvements in performance variables including average power output. In addition, a significant difference was found between the mean power outputs observed between HDC and LDC conditions in favor of HDC. The 1.75% difference between the mean power outputs suggests that the effect of high-dose caffeine intake on performance is dose-dependent, similar to our findings in time trial performance. Although the significant difference between HDC and LDC is within the natural measurement variability limit of the test, it has practical importance in time trial rowing. The findings of our research are consistent with the literature (Anderson et al., 2000; Bruce et al., 2000; Wiles et al., 2006). According to the analyses of the current study, the improvements in power observed after caffeine ingestion are similar to those reported for elite rowers by Bruce et al. (2000) (mean power increase of 2.7% in the 2000 m rowing test). In another study evaluating the 100m time trial performance of swimmers, 5 mg/kg caffeine intake shortened both the completion time and increased the average power production (3.6%) (Wiles et al., 2006). Finally, in our study, participants were classified as habitual moderate caffeine consumers (303.62 mg [SD = 148.34]) (Filip et al., 2020). LDC intake, which corresponds to below the average daily caffeine consumption of the participants, did not have a significant effect on average power compared to PLA. This may be due to the dose being insufficient to increase performance. Considering the time trial times and power outputs, it can be said that caffeine intake improves rowing performance. These results are consistent with the findings of a meta-analysis examining the effect of caffeine on time trial performance in cyclists and using completion time and average power output as performance measures (Chen et al., 2024). Meta-analysis has shown that moderate doses of caffeine (4-6 mg/kg), which is determined to be the optimal dose range, significantly improve time trial performance in cyclists, whereas low doses (1-3 mg/kg) do not provide the same improvement.

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333 In the current study, when HDC and MDC caffeine intakes were compared with PLA, significant 334 differences in mean heart rate were associated with small and moderate increases. It is known that the 335 autonomic nervous system, which is managed by the central nervous system, plays an important role in controlling heart rate (Ginty et al., 2017; Quinlan et al., 1997). Considering the effect of caffeine on 336 337 the central nervous system, it is reasonable that it plays a role in changes in heart rate (Benjamim et al., 2020). Accordingly, the heart rate increases observed in HDC and MDC conditions support the 338 339 known sympathetic stimulating effect of caffeine. This finding shows that the load on the 340 cardiovascular system increases simultaneously with the increase in performance. Although an 341 increase in heart rate during exercise is a physiologically expected result, this may lead to tolerance 342 problems in some individuals during submaximal exercises. Contrary to the current research findings, 343 Bruce et al. (2000) did not observe a significant difference in heart rate during exercise after 6 and 9 344 mg/kg caffeine intake. The contradictory physiological responses to caffeine and exercise may be due 345 to multiple factors, including timing of measurement, individual metabolic differences, and consumption habits (Aguilar-Navarro et al., 2019; Pickering & Kiely, 2019). In the relevant study, no 346 347 information was provided regarding the caffeine use history of the sample group. In addition, although 348 it was stated that blood samples were taken 45 minutes after caffeine intake and a warm-up protocol 349 was applied, it was not clearly stated when the main performance test was started.

Despite the potential of caffeine to enhance performance, sleep deprivation, which poses a risk of performance deficiencies, is among its known side effects (Hindmarch et al., 2000; Youngstedt et al., 2000). Drake et al. (2013) stated that even 400 mg of caffeine taken 6 hours before bedtime significantly reduced sleep quality. Furthermore, it is known that caffeine (200 mg, 3+3 mg/kg, 6 mg/kg), especially in the afternoon, both before and without exercise, causes deterioration in sleep-related parameters (Drapeau et al., 2006; Miller et al., 2014; Ramos-Campo et al., 2019). The findings

of the current study, in which the test sessions started between 19:00 and 20:00, support the literature. Caffeine consumption was found to have a significantly deteriorating effect on sleep quality in the following night in HDC and MDC conditions compared to PLA. Although the pharmacokinetics of caffeine vary between individuals (Temple et al., 2017), it generally has a half-life ranging from 2 to 10 hours (Snel & Lorist, 2011). Although the plasma concentration of caffeine decreases over time, it is likely that it will continue to have a stimulating effect until it is completely eliminated, thus negatively affecting sleep quality. Although sleep quality was assessed only via a scale in the current study, Ali et al. (2015) showed that VO₂, RER, and heart rate values measured during sleep remained high after caffeine intake. These findings support the idea that caffeine may impair sleep quality by maintaining metabolic stimulation even during sleep. In addition, although the deterioration in sleep quality in LDC did not reach statistical significance in our study, the trend was negative. Although caffeine taken in the evening can disrupt all types of sleep parameters through 6-sulfatoxymelatonin (the main metabolite of melatonin) (Shilo et al., 2002) and adrenaline and noradrenaline stimulation in the adrenal medulla (Sawyer et al., 1982; Zhang et al., 2020), the majority of sleep disruptions are thought to be dose-dependent (Robillard et al., 2015). Karacan et al. (1976) reported that among the caffeine doses given 30 minutes before bedtime, caffeine equivalent to 4 cups of coffee disrupted total sleep time, emphasizing that the effect was dose-related. In parallel with our findings, a study conducted with highly trained judokas reported that low-dose (3 mg/kg) caffeine intake administered before evening training did not cause a significant deterioration in objective sleep quality (Filip-Stachnik, 2022). In contrast, Miller et al. (2014) concluded that 3 mg/kg caffeine caused significant sleep disruption. However, in this study, participants did not do any exercise after caffeine intake. It is known that acute exercise improves both sleep quality and sleep latency (Driver & Taylor, 2000; Youngstedt, 2005). In the current study, the negative effect of low-dose caffeine on sleep disruption may have been inhibited thanks to this known benefit of exercise. In addition, although not significant, the tendency of low-dose caffeine to impair sleep quality indicates that individual sensitivity and tolerance levels should be taken into account (Aguilar-Navarro et al., 2019). Although low-dose caffeine did not provide a significant improvement in 100m time trial performances in swimmers, the fact that effect size analyses showed that it may negatively affect sleep quality in some parameters (total sleep time -32 minutes; time to fall asleep +14 minutes) suggests that individual conditions should be evaluated well.

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When daytime sleepiness data are examined, it is remarkable that there is a significant increase in HDC and MDC conditions compared to PLA. This observed increase may be associated with the decrease in the quality of nighttime sleep at the same doses. Laboratory studies show that an average decrease of 90 minutes in nighttime sleep can lead to a decrease in objective alertness the next day by approximately one-third (Bonnet & Arand, 2010). Moreover, sleep deprivation causes significant decreases in cognitive functions such as cognitive and psychomotor reaction speed in the following days (Goel et al., 2009). This may be a limiting factor in terms of the sustainability of performance. It has been reported that even one night of disrupted sleep negatively affects performance in the next competition or training (Oliver et al., 2009; Skein et al., 2011). Therefore, it is important to be aware of the potential risks of caffeine use, which is well known to have negative effects on sleep.

Lastly, when evaluated in terms of side effects, the current study reported that after the HDC condition, effects such as headache, gastrointestinal problems, frequent urination, and insomnia increased significantly. Similar to the findings of our study related to LDC, in the study of Newbury et al. (2022), no significant difference was found in self-reported physical or psychological side effects of 3 mg/kg caffeine compared to placebo.

401 The current study has several strengths. It is one of the few studies that examines the effects of pre-402 exercise caffeine use on both performance and sleep. Furthermore, it is one of the studies that can lead to the determination of the most appropriate supplementation amount by comparing different doses. 403 404 Reliable and reproducible data were provided through the analyses. However, certain limitations 405 should be acknowledged. Since plasma caffeine concentrations were not measured, it was not possible 406 to determine to what extent the observed sleep disturbances were related to this variable. Additionally, 407 sleep parameters were assessed only through self-reporting. However, individual differences could not 408 be taken into account because the participants' habitual sleep patterns were not monitored. Finally, the 409 sample group consisting of trained individuals with moderate caffeine consumption habits limits its 410 generalizability to different training and caffeine sensitivity levels.

5 Conclusion

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412 In conclusion, the effects of different doses of caffeine taken before exercise in the evening on rowing 413 performance against time, cardiovascular responses, sleep quality and daytime alertness were 414 investigated in the present study. The caffeine dose consumed is a determining factor on the time trial 415 performance in the 2000m rowing ergometer. The research findings showed that high and medium doses of caffeine supplementation caused performance improvement compared to placebo in the time 416 417 trial test, while low dose caffeine dose did not show a significant difference. However, the observed 418 improvement was close to significance. Similarly, it was seen that high and medium doses provided a 419 significant improvement compared to placebo in the mean power output findings. In addition, high 420 dose caffeine supplementation caused a significant increase compared to low dose. When the mean 421 heart rates monitored during the test were examined, a significant difference was observed only 422 between high dose and placebo. Finally, it was observed that high and medium doses significantly 423 impaired sleep quality compared to placebo in the night sleep following caffeine use. Low dose 424 caffeine, on the other hand, did not show a significant result despite its tendency to disrupt sleep. It has 425 been proven that athletes' sleepiness the day after caffeine use was significantly higher in high and 426 medium dose conditions.

In summary, it is shown that high-dose caffeine consumption in the evening hours leads to an improvement in performance, but also brings about negative situations such as sleep problems and physical side effects. Athletes should consider the advantages and disadvantages when choosing a supplemental dose. When the research findings are examined, the MDC condition stands out as a balanced option in practical applications due to its limited negative effects on sleep, low side effect reports and performance improvement. Future studies can examine the effects of different doses of caffeine taken at different times of the day on sleep variables. In addition, in our study, sleep variables were assessed only with subjective scales. Studies using actigraphy or polysomnography can be designed. Although the sleepiness of the athletes the next day was evaluated, how caffeine-related sleep deprivation affects performance, especially in athletes competing on consecutive days, can be an interesting research topic. Finally, genetic metabolism differences (such as CYP1A2 genotype) of the participants can be analyzed to determine their sensitivity to caffeine in terms of sleep and contribute to making comments at an individual level.

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