



Acute Effects of Caffeine Supplementation During a 20-Minute Cycling Time Trial in Amateur Cyclists: A Pilot Randomized Crossover Study

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Abstract:

Background: Caffeine is commonly used as an ergogenic aid to enhance athletic performance; however, evidence regarding its effectiveness during cycling remains equivocal, particularly when performance is assessed under conditions that closely resemble real-world practice.

Aims: This study examined the acute effects of caffeine ingestion on physiological and performance outcomes during a 20-minute self-paced cycling time trial in amateur cyclists. Additionally, inter-individual variability in performance responses to caffeine supplementation was explored.

Methods: Eight recreationally trained male cyclists participated in a randomized, double-blind, placebo-controlled crossover pilot study. Experimental sessions were separated by a seven-day washout period. Participants ingested either caffeine (6 mg·kg⁻¹ body mass) or a placebo 60 minutes before completing a 20-minute self-paced time trial using their personal bicycles mounted on a smart trainer. Mean power output, total distance covered, and heart rate were recorded throughout the test. Individual performance responses were also evaluated.

Results: No statistically significant differences were identified between caffeine and placebo conditions for heart rate, mean power output, or distance covered ($p > 0.05$ for all comparisons). Despite the absence of significant group-level effects, considerable inter-individual variability in percentage changes in mean power output was observed. In the exploratory analysis, three participants (37.5%) showed increases $\geq 2\%$, four (50.0%) showed changes between -2% and $+2\%$, and one (12.5%) showed a decrease $\leq -2\%$. Given the absence of repeated baseline measurements to estimate measurement error and day-to-day variability, these individual changes should not be interpreted as evidence of confirmed responsiveness to caffeine. Mild adverse effects, including increased perception of heart rate and feelings of nervousness, were reported by a subset of participants.

Conclusion: Ingestion of 6 mg·kg⁻¹ of caffeine did not significantly influence physiological responses or cycling performance during a 20-minute self-paced time trial in this sample of amateur cyclists. Individual percentage changes varied across participants; however, these exploratory observations cannot establish true individual responsiveness. Larger studies incorporating repeated measurements are required to determine whether such variability reflects genuine differences in caffeine response.

Keywords: Caffeine; Cycling; Endurance performance; Ergogenic aids; Individual responsiveness

1. INTRODUCTION

Caffeine remains one of the most frequently consumed nutritional ergogenic aids among athletes and recreationally active individuals (Close et al., 2016; Fałczyńska et al., 2026). Its popularity is largely attributed to its potential to

enhance exercise performance through multiple physiological pathways involving both the central nervous system and peripheral tissues (Wan et al., 2017; Qiu et al., 2025). By blocking adenosine receptors, caffeine can increase neural excitability, promote alertness, and modify perceptions related to fatigue and effort during exercise (Guest et al., 2021; Temple et al., 2017).

As a result of these physiological actions, caffeine supplementation has been extensively investigated across a wide variety of exercise modalities (Kerksick et al., 2018; Martins et al., 2020; Pickering & Grgić, 2019). Current evidence generally supports its ability to produce modest improvements in endurance-based activities, repeated high-intensity efforts, and several performance-related outcomes, although the magnitude of these effects varies considerably

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between individuals and exercise protocols (Chen et al., 2024; Guest et al., 2021).

Within cycling, caffeine has frequently been examined as a strategy to improve time-trial performance and power production (Felippe et al., 2018; Glaister et al., 2016; Kerksick et al., 2018). Nevertheless, findings remain inconsistent, with some investigations reporting meaningful ergogenic benefits while others demonstrate little or no performance enhancement (Chen et al., 2024; Southward et al., 2018). Such discrepancies may be partially explained by differences in participant characteristics, habitual caffeine consumption, exercise protocols, and individual biological factors that influence responsiveness to caffeine ingestion (Pickering & Kiely, 2018; Silva et al., 2025).

Most investigations examining caffeine supplementation in cycling have been conducted using laboratory-based cycle ergometers under highly controlled conditions (Guest et al., 2021; Chen et al., 2024; Wu et al., 2026; Xue et al., 2025). Although these experimental models provide strong internal validity, they may not adequately reflect real-world cycling practice, where performance is influenced by pacing strategies, equipment familiarity, and individual biomechanics (Leo et al., 2021; Miller, 2021). Despite the extensive literature on caffeine supplementation in cycling, limited evidence is available regarding its effects during self-paced cycling assessments performed on athletes' own bicycles mounted on smart trainers, a setting that more closely resembles real-world training and competition environments (Trujillo-Colmena et al., 2024; Davenport et al., 2020). Furthermore, relatively few studies have explored the degree of inter-individual variability in ergogenic responses under ecologically valid conditions.

Therefore, the present pilot study aimed to investigate group-level effects and explore inter-individual variability in performance responses to caffeine ingestion during a 20-minute self-paced cycling time trial.

2. MATERIAL AND METHOD

2.1 Study Design

A randomized, double-blind, placebo-controlled crossover approach was adopted for this pilot investigation. Each cyclist completed two experimental sessions in a counterbalanced order, receiving caffeine (CAF) during one trial and

placebo (PLA) during the other. A seven-day interval separated the experimental visits to minimize residual effects of supplementation. The allocation sequence was generated electronically prior to data collection, and both participants and researchers remained unaware of the treatment administered until completion of all analyses.

2.2 Participants

Eight male amateur cyclists completed the study. Participant characteristics were as follows: age 29.8 ± 9.6 years, body mass 76.5 ± 11.6 kg, height 1.79 ± 0.06 m, body mass index 24.1 ± 2.7 kg·m⁻², and habitual caffeine intake 174.0 ± 42.9 mg·day⁻¹. Based on their daily caffeine consumption, participants were classified as moderate habitual caffeine consumers. Habitual caffeine intake was assessed by a registered nutritionist using the Gonçalves Caffeine Intake Questionnaire (Gonçalves et al., 2017). In addition, a 24-hour dietary recall was obtained before the first experimental trial. Participants received a copy of their dietary record and were instructed to replicate the same food and beverage intake before the second experimental session to minimize nutritional variability between conditions.

Eligibility criteria included: (i) at least six months of cycling training experience; (ii) a minimum training frequency of two sessions per week; and (iii) familiarity with indoor cycling. Exclusion criteria included cardiovascular disease, hypertension, gastrointestinal disorders, use of medications known to affect cardiovascular or metabolic responses, and lower-limb musculoskeletal injuries that could impair exercise performance. All participants provided written informed consent prior to participation. The study was conducted in accordance with the Declaration of Helsinki and approved by the local Institutional Research Ethics Committee (approval number: 2.540.958). Participants were instructed to abstain from caffeine consumption, alcohol intake, and strenuous physical exercise for 24 hours before each experimental session. They were also asked to maintain similar dietary habits before both trials.

2.3 Experimental Procedures

Participants visited the laboratory on three occasions. During the initial visit, each cyclist's personal bicycle was installed on an interactive smart trainer (Elite Suito-T, Elite Srl, Fontaniva, Italy). Bicycle configuration variables, including saddle and handlebar positioning, were documented and replicated in subsequent sessions.



Participants also completed a familiarization 20-minute self-paced cycling time trial to reduce potential learning effects. The remaining two visits corresponded to the experimental conditions and were separated by a seven-day washout period. Throughout this interval, participants were instructed to maintain their habitual training routines and avoid substantial modifications in training load, intensity, or frequency. Upon arrival for each experimental session, participants rested quietly in a seated position for 10 minutes. They then consumed either caffeine (6 mg·kg⁻¹ body mass) or placebo with 250 mL of water. Following ingestion, a 60-minute waiting period was observed to allow sufficient time for absorption. After supplementation, cyclists completed a

standardized 5-minute warm-up at a self-selected low intensity.

The experimental task consisted of a 20-minute self-paced cycling time trial performed at the highest sustainable effort. All tests were conducted using the participants' own bicycles mounted on the smart trainer. Throughout the trial, participants remained seated and received only information regarding elapsed time. No additional performance feedback was provided. Mean power output (W), total distance covered (km), and heart rate (beats·min⁻¹) were recorded continuously. Heart rate data were collected using a Polar H9 monitor (Polar Electro Oy, Kempele, Finland) and exported for subsequent analysis.

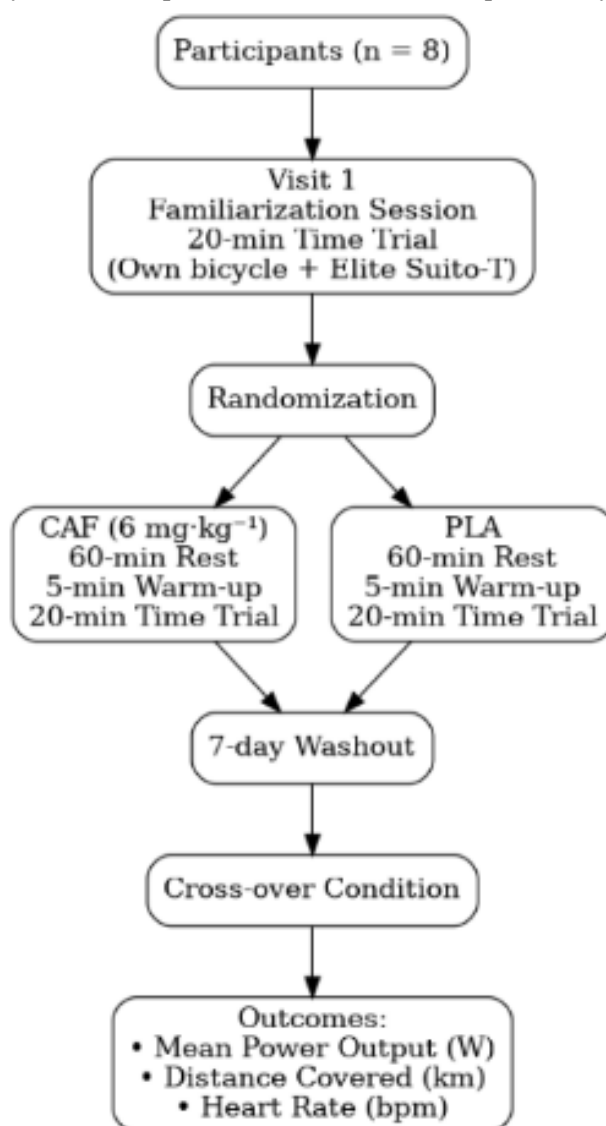


Figure 1. Schematic representation of the experimental design. Participants completed a familiarization session followed by two randomized, double-blind, placebo-controlled crossover trials separated by a 7-day washout period. Physiological and performance outcomes were assessed during a 20-minute self-paced cycling time trial.

2.4 Supplementation, Randomization, and Blinding

The supplementation protocol consisted of either caffeine (6 mg·kg⁻¹ body mass) or placebo (microcrystalline cellulose), both provided in visually identical gelatin capsules. Capsules were ingested with 250 mL of water approximately 60 minutes before exercise testing. The selected caffeine dose was based on previous evidence indicating ergogenic effects within the moderate-to-high supplementation range (Mor et al., 2024; Guest et al., 2021). Treatment order was established using a computerized randomization procedure performed before participant enrollment. Allocation concealment was ensured through sealed opaque envelopes prepared by a researcher who was not involved in data collection or outcome assessment. Blinding was maintained throughout the study, with neither participants nor investigators aware of the supplementation condition until all data analyses had been completed.

2.5 Blinding Effectiveness and Side Effects Assessment

Blinding effectiveness was assessed immediately after each experimental trial. Participants were asked to identify the substance they believed they had ingested (caffeine, placebo, or unknown). Responses were recorded to evaluate the success of the blinding procedures. Potential side effects associated with caffeine ingestion were assessed using a standardized questionnaire administered immediately after each trial. Participants reported the presence and intensity of symptoms, including increased heart rate, agitation or nervousness, headache, dizziness, nausea, and perceived impact on exercise performance. Responses were descriptively analyzed.

2.6 Statistical Analysis

Data were analyzed using GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA). Data normality was assessed using the Shapiro–Wilk test. Paired t-tests were used to compare physiological and performance variables between caffeine and placebo conditions. Statistical significance was set at $p < 0.05$. Results are presented as mean $\pm$ standard deviation (SD). Effect sizes were calculated using Cohen's d_z and interpreted as trivial (<0.20), small (0.20 – 0.49), moderate (0.50 – 0.79), and large (≥ 0.80). Individual responses to caffeine supplementation were explored descriptively by calculating the percentage change in mean power output between

the caffeine and placebo conditions. For descriptive and visualization purposes, individual changes were categorized using $\pm 2\%$ reference thresholds ($\geq 2\%$ improvement, -2% to $+2\%$, and $< -2\%$), based on previous literature examining inter-individual responses to ergogenic aids (Grgic, 2018; Xue et al., 2025). However, because repeated baseline measurements were not available to estimate within-subject measurement error and normal day-to-day variability, these thresholds were used solely as exploratory reference values and were not considered evidence of confirmed responder, non-responder, or negative-responder status.

3. RESULTS AND DISCUSSION

3.1 Heart Rate

Mean heart rate was higher in the caffeine condition compared with placebo (CAF: 181.6 ± 8.9 bpm; PLA: 178.6 ± 6.8 bpm). However, this difference was not statistically significant (mean difference: 3.0 bpm; 95% CI: -0.69 to 6.69 ; $p = 0.096$). The effect size was moderate (Cohen's $d_z = 0.68$).

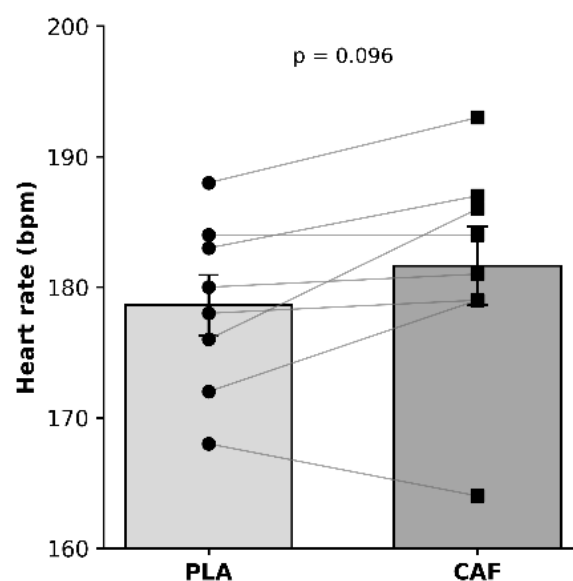


Figure 2. Comparison of mean heart rate between placebo (PLA) and caffeine (CAF) conditions. Data are presented as mean $\pm$ SEM with individual paired responses.

Although mean heart rate was numerically higher following caffeine ingestion, no statistically significant difference was observed between conditions. Caffeine primarily acts through adenosine receptor antagonism, which may increase sympathetic nervous system activity and catecholamine release (Temple et al., 2017; Guest



et al., 2021; Negaresh et al., 2026). Previous studies have reported increased heart rate responses during moderate-to-high-intensity exercise following caffeine ingestion (Delleli et al., 2026; Negaresh et al., 2026). However, the present findings suggest that caffeine supplementation did not significantly alter mean heart rate during the 20-minute self-paced cycling time trial in this sample of amateur cyclists.

3.2 Power Output

Mean power output was slightly higher after caffeine ingestion compared with placebo (CAF: 314.3 ± 27.1 W; PLA: 308.6 ± 26.1 W). However, this difference was not statistically significant (mean difference: 5.63 W; 95% CI: -1.51 to 12.76; $p = 0.105$). The effect size was moderate (Cohen's $d_z = 0.66$).

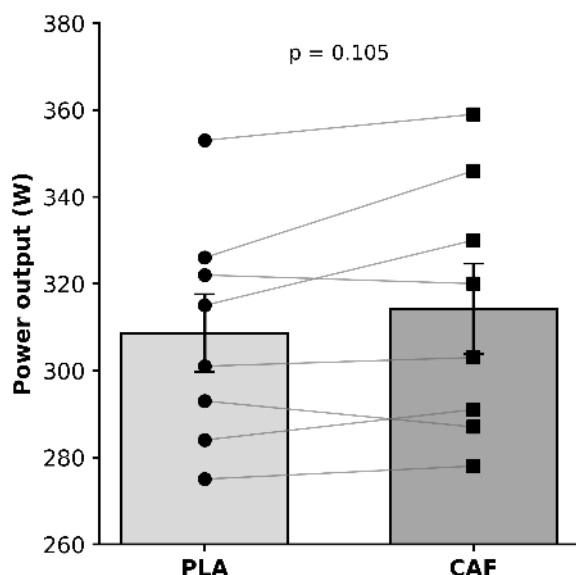


Figure 3. Comparison of mean power output between placebo (PLA) and caffeine (CAF) conditions. Data are presented as mean $\pm$ SEM with individual paired responses.

Although caffeine ingestion resulted in a modest numerical increase in mean power output, the difference between conditions was not statistically significant. The confidence interval included zero, indicating uncertainty regarding the presence of an ergogenic effect at the group level. Despite the moderate effect size (Cohen's $d_z = 0.66$), the small sample size and statistical uncertainty preclude definitive conclusions regarding the effects of caffeine on power output.

These findings contrast with recent systematic reviews and meta-analyses reporting small but positive effects of caffeine on cycling performance (Chen et al., 2024; Wu et al., 2026). However, they are consistent with studies involving recreationally trained individuals and self-paced exercise protocols, in which performance responses appear more variable (Silva et al., 2025; Bezuglov et al., 2025).

Differences in caffeine dosage may partly explain these findings. Previous evidence suggests that lower caffeine doses may provide comparable performance benefits in certain cycling contexts while potentially reducing adverse effects (Burke, 2017; Xue et al., 2025). Therefore, the dose administered in the present study may not have been optimal for all participants.

From a practical perspective, these findings do not support assuming a consistent ergogenic effect of caffeine supplementation across individuals. Although individual percentage changes in power output varied, these exploratory observations cannot establish true individual responsiveness because measurement error and day-to-day variability were not quantified. Future studies incorporating repeated measurements and larger samples are needed to clarify the sources of this variability.

3.3 Distance Covered

Distance covered during the 20-minute time trial did not differ significantly between caffeine and placebo conditions. Participants covered 7.89 ± 0.52 km in the placebo condition and 7.97 ± 0.54 km in the caffeine condition, corresponding to a mean difference of approximately 0.08 km ($p = 0.455$). Although distance was numerically greater following caffeine ingestion, this difference was not statistically significant.

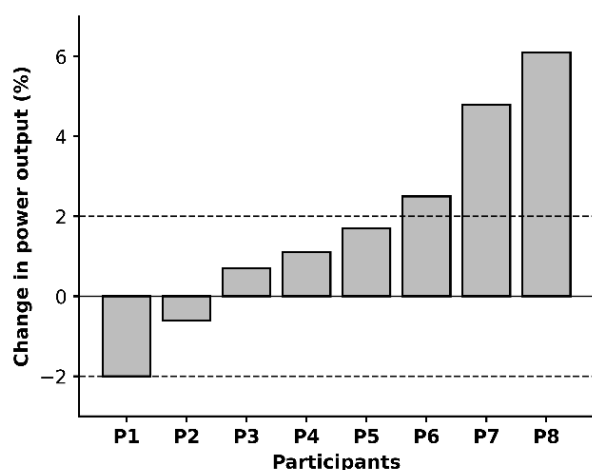


Figure 4. Individual percentage changes in mean power output following caffeine ingestion compared with placebo during the 20-minute time trial. Dashed lines indicate the $\pm 2\%$ reference thresholds used for descriptive visualization of individual changes. These thresholds were not used to infer confirmed responder or non-responder status.

An important consideration when interpreting these findings is the methodological distinction between the present study and previous investigations of caffeine supplementation in cycling. Most studies have employed laboratory-based cycle ergometers under fixed or semi-controlled conditions (Guest et al., 2021; Chen et al., 2024; Wu et al., 2026; Xue et al., 2025). Although these approaches provide substantial experimental control, they may not fully reproduce cyclists' habitual biomechanics, pacing strategies, and equipment interactions. In contrast, the present study used participants' own bicycles mounted on smart trainers, preserving familiar cycling positions and pedaling mechanics.

This approach may provide greater ecological validity for self-paced cycling assessments, in which performance depends on pacing strategies and sustained effort (Barsumyan et al., 2025; Borszcz et al., 2018; McGrath et al., 2019). Therefore, differences in experimental protocols may partly explain discrepancies between the present findings and previous research. However, the influence of these methodological differences remains speculative and requires further investigation.

3.4 Exploratory Analysis of Individual Performance Changes

Considerable inter-individual variability was observed in percentage changes in mean power

output following caffeine ingestion. Three participants (37.5%) showed increases $\geq 2\%$, four (50.0%) showed changes between -2% and $+2\%$, and one participant (12.5%) showed a decrease $< -2\%$. These categories were used exclusively for descriptive purposes and should not be interpreted as evidence of confirmed individual responsiveness to caffeine.

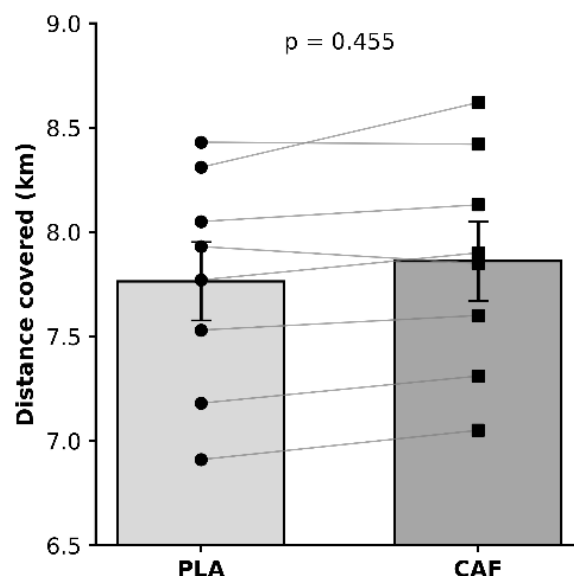


Figure 5. Comparison of distance covered between placebo (PLA) and caffeine (CAF) conditions. Data are presented as mean $\pm$ SEM with individual paired responses.

An exploratory finding of the present study was the variability observed in individual percentage changes in mean power output following caffeine supplementation. Despite the absence of significant group-level differences in power output and distance covered, individual changes varied across participants. However, these observations should be interpreted cautiously because measurement error and normal day-to-day variability were not quantified. Grgic (2018) argued that the identification of non-responders may be influenced by factors such as measurement error, exercise modality, caffeine dose, and day-to-day variability in performance responses. Davison et al. (2025) demonstrated that individuals carrying the ADORA2A TT genotype exhibited greater physiological responses following caffeine ingestion, supporting the hypothesis that genetic factors may contribute to inter-individual variability in physiological responses to caffeine.

The blinding procedures adopted in the present study appeared to be generally effective, as most participants were unable to correctly identify the



experimental condition. Specifically, six out of eight participants reported uncertainty regarding whether they had ingested caffeine or placebo, reducing the likelihood that expectancy effects substantially influenced the observed outcomes. This finding is particularly relevant considering the growing body of evidence supporting placebo and nocebo responses associated with caffeine supplementation in sport settings (Saunders et al., 2017; Vega-Muñoz et al., 2024).

Because repeated baseline measurements were not performed, the observed individual differences should be considered exploratory rather than evidence of distinct responder phenotypes. Future studies incorporating repeated assessments, larger samples, and potential moderating factors, such as genetic profile, habitual caffeine intake, and sleep characteristics, are needed to better understand inter-individual variability in responses to caffeine supplementation.

3.5 Blinding Effectiveness

Blinding effectiveness was assessed following each experimental trial. Two participants (25.0%) correctly identified the caffeine condition, whereas six participants (75.0%) reported being unable to determine which substance had been ingested. No participants incorrectly identified the experimental condition. These findings suggest that the blinding procedures were generally effective in minimizing expectancy bias.

3.6 Side Effects

Three participants (37.5%) reported mild adverse effects following caffeine ingestion. The most commonly reported symptom was an increased awareness of heart rate ($n = 2$), while one participant reported agitation or nervousness ($n = 1$). Furthermore, three participants indicated that caffeine was perceived to have a negative influence on their performance during the time trial. No serious adverse events were observed.

Regarding tolerability, caffeine supplementation was generally well tolerated. Nevertheless, a small number of participants reported mild adverse effects, primarily an increased awareness of heart rate and feelings of agitation or nervousness (Raya-González et al., 2019; Szerej et al., 2024). Furthermore, three participants perceived that caffeine negatively influenced their performance during the time trial. These findings align with recent evidence indicating that acute caffeine

ingestion increases the likelihood of side effects in athletes in a dose-dependent manner (Filip-Stachnik et al., 2021; Negaresh et al., 2026). Interestingly, our findings differ from those reported by Bezuglov et al. (2025), who observed no effect of acute caffeine ingestion on heart rate responses or perceived exertion in young elite soccer players. Collectively, these findings suggest that individual tolerability should be considered when interpreting the effects of caffeine supplementation. However, the present pilot study does not provide sufficient evidence to support individualized supplementation strategies.

The findings of this study should be interpreted in light of several limitations. The small sample size, inherent to its pilot design, limits the generalizability of the results and may have reduced statistical power to detect small performance effects. Additionally, genetic factors and sleep quality were not controlled, and the potential influence of habitual caffeine intake cannot be ruled out. The absence of repeated baseline trials also prevented the estimation of within-subject measurement error and day-to-day performance variability. Nevertheless, the randomized, double-blind, crossover design and the use of participants' own bicycles mounted on smart trainers represent important methodological strengths. By examining caffeine supplementation during a 20-minute self-paced cycling time trial, this study provides preliminary evidence regarding performance outcomes and exploratory inter-individual variability under conditions that more closely resemble habitual cycling practice.

4. CONCLUSION

In this pilot randomized crossover study, acute supplementation with $6 \text{ mg} \cdot \text{kg}^{-1}$ of caffeine did not significantly affect mean heart rate, power output, or distance covered during a 20-minute self-paced cycling time trial in amateur cyclists. Although individual percentage changes in power output varied, these exploratory observations cannot establish true individual responsiveness because within-subject measurement error and day-to-day variability were not quantified. Therefore, the findings provide insufficient evidence of an ergogenic effect of caffeine under the conditions investigated. Larger studies incorporating repeated measurements and potential moderating factors, such as genetic characteristics, habitual caffeine intake, and sleep-related variables, are warranted to

further investigate individual variability in caffeine responses during cycling performance.

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6. AUTHOR CONTRIBUTION STATEMENT

LR, AS, and AM conceived and designed the study. LR and AS conducted data collection. LR, MM, VM, and AM contributed to data analysis and interpretation. LR drafted the initial version of the manuscript. MM, VM, and AM critically revised the manuscript for important intellectual content. All authors reviewed and approved the final version of the manuscript.

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