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ORIGINAL ARTICLE

Caffeine supplementation for 4-day, followed by acute ingestion, did not impact triathlete output power after submaximal intensity exercise

A suplementação de cafeína por 4 dias, seguida de ingestão aguda, não impactou na potência de triatletas após realizarem exercício de intensidade submáxima

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Abstract

Introduction: The aim of this study was to test the hypothesis that caffeine supplementation (6 mg·kg^{-1} body mass) for 4-days, followed by acute intake, would impact five male triathletes output power after performed submaximal intensity exercise. *Methods*: This was a randomized, double-blind, placebo-controlled crossover study, placebo (4-days) - placebo (acute) PP, placebo (4-day) -caffeine (acute) PC, and caffeine (4-day) - caffeine (acute) CC. Participants abstained from dietary caffeine sources for 4 days and ingested capsules containing either placebo or caffeine (6 mg.kg^{-1} body mass day in one absorption). The acute trials the capsules containing placebo or caffeine (6 mg·kg^{-1} body mass day in one absorption) were ingested 60min before completing exercise in a treadmill for 40min (80% VO_2max) and to perform the Wingate test. *Results*: Blood lactate was determined before, 60min after ingestion, and immediately after the exercise on the treadmill, the Wingate test, and after the recovery (10-min). CC and PC trials did not change the cardiopulmonary variables (P>0.05) and the anaerobic power variables (peak/mean power output and fatigue index) (P>0.05). The PC trial compared with PP promoted improvements in the curve power output in 2 sec by 31.19% (large effect-size d = 1.08; P<0.05) and 3 sec by 20% (large effect-size d = 1.19; P<0.05). A 10min recovery was not sufficient to reduce blood lactate concentration in the PC trial compared with PP (PC, 13.73±2.66 vs. PP, 10.26±1.60 mmol.L^{-1}; P<0.05, respectively) (P<0.05). *Conclusion*: In conclusion, these results indicate that caffeine supplementation (6 mg·kg^{-1} body mass) for 4 days, followed by acute ingestion, did not impact the triathletes output power after performed submaximal intensity exercise. Nutritional interventions may help researchers and athletes to adapt strategies for manipulating caffeine use.
Key-words: caffeine metabolism, Wingate test, blood lactate, performance.

Resumo

Objetivo: O objetivo deste estudo foi testar a hipótese de que a suplementação de cafeína (6 mg · kg^{-1} de massa corporal) por 4 dias, seguida de ingestão aguda, afetaria a potência de cinco triatletas masculinos após realizarem o exercício de intensidade submáxima. *Métodos*: Foi realizado um estudo cruzado, randomizado, duplo-cego, placebo-controlado, placebo (4-dias) - placebo (agudo) PP, placebo (4-dias) - cafeína (agudo) PC, cafeína (4-dias) - cafeína (agudo) CC. Os participantes se abstiveram de fontes alimentares de cafeína por 4 dias e ingeriram cápsulas contendo placebo ou cafeína (6 mg.kg^{-1} de massa corporal por dia). Os participantes nos ensaios agudos ingeriram cápsulas contendo placebo ou cafeína (6 mg.kg^{-1}de massa corporal) 60 minutos antes de realizar o exercício na esteira por 40 minutos (80% VO_2máx) e em seguida realizaram o teste de Wingate. O lactato sanguíneo foi determinado antes, 60 minutos após a ingestão, imediatamente após o exercício na esteira e no teste de Wingate e, após 10 min. de recuperação. *Resultados*: Nos ensaios CC e PC não foram observadas alterações nas variáveis cardiopulmonares (P>0,05) e nas variáveis de potência anaeróbica (potência de pico, potência média e índice de fadiga) (P>0,05). No ensaio PC comparado ao PP promoveu melhorias na curva de potência no tempo de 2 segundos (31,19% - *large effect-size* d = 1,08; P<0,05) e no tempo de 3 segundos (20% - large effect-sized = 1,19; P<0,05). A recuperação de 10 minutos não foi suficiente para reduzir a concentração de lactato sanguíneo no ensaio PC em comparação ao PP (PC, 13,73 ± 2,66 vs. PP, 10,26 ± 1,60 mmol.L^{-1}; P<0,05, respectivamente) (P<0,05). *Conclusão*: Em conclusão, esses resultados indicam que a suplementação de cafeína (6 mg·kg-1 de massa corporal) por 4 dias, seguida de ingestão aguda não impactou na potência de saída de triatletas após realizarem o exercício de intensidade submáxima. Intervenções nutricionais podem auxiliar pesquisadores e atletas a adaptarem estratégias na manipulação do uso de cafeína.

Palavras-chave: metabolismo de cafeína, teste de Wingate, lactato sanguíneo, performance.

Introduction

Several physiological attributes contribute to the success of endurance exercises [1], including the interaction between the realization of submaximal (i.e., ≤ 99% of VO_2max) and maximal exercises [2]. To date, a wide range of acute and chronic interventions have been investigated regarding performance improvement in endurance exercises [3,4]. Caffeine (1,3,7-trimethylxanthine) has been described as an effective ergogenic aid for enhancing performance in various sports [5-10]. Many researchers [11,12] have argued that the primary focus behind the ventilatory effect (i.e., increased alveolar ventilation) of caffeine is the central stimulation of the respiratory medullary complex. The explanations proposed by Chapman and Stager [12] are that the acute use of caffeine significant increase in minute ventilation (VE) during submaximal exercise, and this result in a rise in improving arterial oxyhemoglobin saturation (SaO2) and oxygen delivery to the working musculature. Furthermore, the acute use of caffeine may improve the coupling excitation/contraction by facilitating Ca+ exchange of the sarcoplasmic reticulum and/or by increasing the myofibrillar sensitivity for this ion [9]. This would result in positive changes in the power parameters of athletes [9,10].

To determine the ideal conditions for maximizing the physiological effects of caffeine, source [13], the timing of intake [14] and habituation [15] have been investigated. Chronic habituation to caffeine impacts the concentration of A1 and A2A receptors in various brain regions [16,17] and expression of A2A/A2B receptors distributed in the sarcolemma of skeletal muscle [18].

This includes A2A expression in the striatum, a subcortical region essential for coordinating motor activity [19], and oxygen consumption [4]. Moreover, the expression of A2A/A2B receptors of skeletal muscle is involved in the regulation of contractility of type I and type II fibers [18]. On this premise, it has been speculated whether caffeine intake exceeding usual consumption for a shorter period (i.e., four days) could lead to tolerance to the ergogenic effects of caffeine already demonstrated for long periods (≥ 20 days) [4,17].

The aim of this study was to test the hypothesis that caffeine supplementation (6 mg·kg-1 body mass) for 4-days, followed by acute intake would impact output power triathletes after performed submaximal intensity exercise.

Methods

Subjects

Five male triathletes federated from the state of Rio de Janeiro, all participants in national competitions in Brazil. The athletes presented (mean ± SD) were aged 33.0 ± 7.4 years, had a body mass of 75.79 ± 8.3 kg, and height of 179.4 ± 4.3 cm. All of them had at least 3.8 ± 1.2 years of experience in the sport. No athlete had a medical history of the cardiopulmonary disease or used any medication during the study. Habitual caffeine consumption was assessed using an adapted version of the Landrum, Meliska and Loke [20] caffeine consumption questionnaire. The athletes were regular consumers of caffeine (242 ± 39 $mg.day^{-1}$) with <300 $mg.day^{-1}$ defined as low habitual caffeine consumption and >300 $mg.day^{-1}$ as high habitual caffeine consumption [21]. In addition, a 24-hour dietary record was completed by each athlete before the first trial, it was then photocopied and handed back to the athletes so that the same diet could be repeated for subsequent trials (daily energy, 4016 ± 1119 kcal; carbohydrate, 52.39 ± 17.26%; protein, 16.51 ± 6.68%; fat, 31.08 ± 10.96%). Energy and macronutrient intake were analyzed by the software Dietpro® 5i (Dietpro, Minas Gerais, Brazil). All participants were notified of the investigation procedures, requirements, benefits, and risks before providing written consent. The protocol (2.540.958/2018) was approved by the Scientific and Ethics Committee of the Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

Study design

This is a randomized, placebo-controlled, double-blind crossover study: placebo (supplementation for 4 days) - placebo (acute supplementation) (PP; n = 5); placebo (supplementation for 4 days) - caffeine (acute supplementation) (PC; n = 5); and caffeine (supplementation for 4 days) - caffeine (acute supplementation) (CC; n = 5). Each athlete visited the laboratory on four occasions. The first visit involved a preliminary confirmation of the maximum exercise capacity on the treadmill (i.e., cardiopulmonary exercise testing) to determine maximal oxygen uptake (VO2max) (Table I), assessment of daily caffeine consumption, familiarization with the protocol in the submaximal exercise at steady-state treadmill, and familiarization with the test in the cycle ergometer (Wingate test). From the first to the fourth day, the athletes were instructed to withdraw all their caffeine consumption (i.e., food sources of caffeine). The athletes were also instructed to continue the routine per week training (Table II). Experimental trials involved supplementation for four days (athletes were instructed to ingest single daily dose at a similar time: 8:00 a.m.) of placebo (250 mg magnesium silicate) or caffeine (6 $mg.kg^{-1}$ body mass) capsules (first, second, third and fourth days). Acute supplementation consisted of ingestion of the capsules of placebo (250 mg magnesium silicate) or caffeine (6 $mg.kg^{-1}$ body mass; the capsules were ingested with 250 ml of water) on the day of the experimental trials in the laboratory (fifth day). During supplementation for four days, the athletes were monitored by telephone contact, e-mail, and in-person [17]. On the second, third, and fourth visits (fifth day, acute supplementation), the athletes arrived at the laboratory (without performing any physical activity 24 h before) fasting (8 h) and subsequently an intravenous cannula (20G Jelco, B. Braun Medical Inc., USA) was then inserted into a forearm, and then a blood sample (10 ml) was obtained before capsule ingestion (Rest). The amount of time to collect each sample was ~ 150 seconds. After ingestion of the capsules, athletes remained in the tests area for 60 min without performing any physical activity, and immediately after the second blood sample was collected (AC). The athletes then underwent a 5-min warm-up on an exercise treadmill (50% VO_2max), and after performing the submaximal exercise in the treadmill for 40 min (80% VO_2max), a blood sample was collected (SE). Then the athletes performed the test on the cycle ergometer, and two blood samples were collected immediately after (WT) and 10 min after (R). During the 10-min recovery, the athletes were lying on a stretcher and did not make any physical effort. Two days of intervals (i.e., washout) between experimental trials were established. The experimental trials were performed at the same time of day (7:00 a.m.). During the experimental trials (fifth day, acute supplementation), the temperature of the laboratory was regulated between 20°C and 22°C (Figure 1).

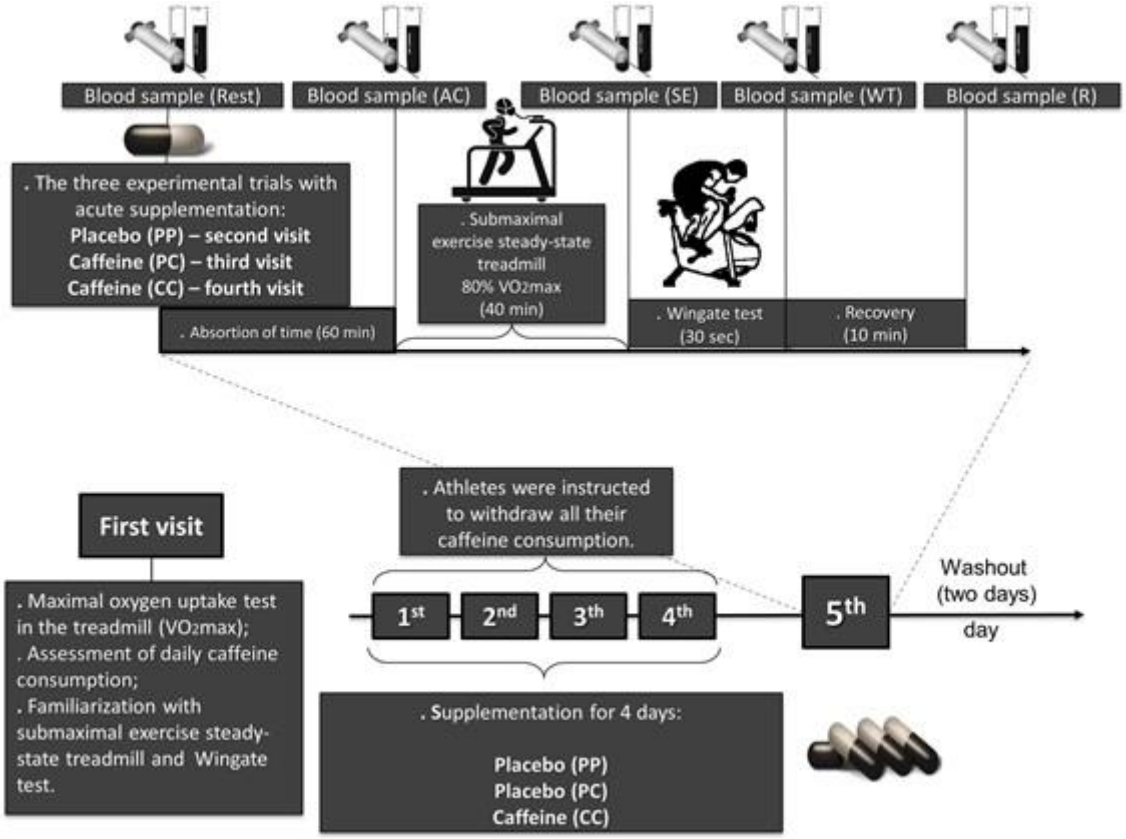

Figure 1 - *Experimental design.*

Maximal oxygen uptake incremental test in the treadmill

All triathletes were assessed using the same ramp protocol on a treadmill (ATL/Inbramed, Porto Alegre, Brazil) programmed to achieve the maximum duration of 8 to 14 min. After a 1-min walk at 5.5 km/h, the velocity was rapidly increased to 6 km/h and then increased by 0.14 km/h every 7.5 s (1.12 km/h every minute), maintaining treadmill inclination of 1% [22]. The heart rate was measured every 3 breaths (breath by breath) from a continuous recording on third derivations (using MC5, V2, and AVF) measured by a digital electrocardiograph ErgoMet (HeartWare, Belo Horizonte, Brazil) with the ErgoMet software version 1.0.3.2 (HeartWare). Ventilatory expired gas was collected with a preVent® pneumotachograph (MedGraphics, St. Paul, MN) with the aid of a neoprene mask and was analyzed by VO2000 (MedGraphics), which was calibrated. The adapted criteria to ensure a maximal exercise test were (a) achievement of maximum voluntary exhaustion, despite verbal encouragement, accompanied by a rate of perceived exertion (adapted Borg scale, 0-10 points) and (b) a respiratory exchange ratio greater than 1.10 [22].

Submaximal exercise steady-state treadmill

After the 5-min warm-up on the treadmill (50% VO_2max), the athletes performed the exercise on a steady-state treadmill with a submaximal protocol of 40 min (80% VO_2max). Athletes were monitored every 5min with the following cardiopulmonary variables: heart rate (HR), maximal oxygen uptake (VO_2), carbon dioxide production (VCO_2), minute volume (VE), O_2 expiration fraction (FeO_2), and rate of perceived exertion (RPE) (adapted Borg scale, 0-10 points).

Inertial Wingate test

After performing the submaximal exercise steady-state treadmill, the triathletes performed the Wingate test. Before the test, the following instructions were given by the investigators: (i) in the first seconds of the test, they should pedal from 0 rpm to the greatest pedaling velocity possible (rpm) for 30 sec; and (ii) maintain this high-power level during the longest possible time until the test end [23]. Two of the authors motivated the subjects during the test duration. The Wingate test was performed in a cycle ergometer of mechanical resistance

(Biotec 2100, Cefise®, São Paulo, Brazil) with double sensors and weights of basket. The resistance was established with each athlete's body mass (0.075 $kg \cdot kg^{-1}$). The cycle ergometer was connected to a laptop using "Ergometric" software (version 7.0, Cefise®) for the collection and storage of data, such as peak power output (Watts), mean power output (Watts), fatigue index (peak power output - minimum power output) / peak power output × 100) (%) and curve power output (Watts).

Blood caffeine and lactate analysis

The measurement of blood levels of caffeine and lactate was performed in Rest, AC, SE, WT, and R. Liquid chromatography was adapted from Ribeiro *et al.* [5]. The liquid chromatographic analyses were carried out using a Shimadzu chromatograph (Shimadzu® Corp., Kyoto, Japan), equipped with an LC-20AT quaternary solvent pump unit, an SPD-M20A diode array detector operating at 274 nm. The caffeine analyses were performed at 35°C using a column oven model CTO-20A, and solvent degassing was performed by a DGU-20A5 degasser. An LCsolution™ software, version 1.25 SP1, was used for system control and data acquisition. The caffeine was extracted from human serum by a protein precipitation procedure. Aliquots of 200 µL of drug-free human serum were spiked with 25 µL of caffeine working solutions or samples obtained from the athletes and were transferred to 10-mL conical glass tubes. Then, 25 µL of pentoxifylline solution (60 µg/mL) was used as internal standard and 300 µL of methanol was added. This mixture was vortex agitated for 1 min and then centrifuged at 3000 rpm for 5 min. Finally, 250 µL of the supernatant was collected, and 50 µL was analyzed by HPLC. The quantification of caffeine in serum was carried out using a calibration curve obtained by spiking aliquots of drug-free serum with working solutions of caffeine at concentrations of 1.2, 3.6, 8, 40, 120, and 240 µg/mL resulting in concentrations of 0.15, 0.45, 1.0, 5, 15, and 30 µg/mL in a drug-free serum. Plotting was performed by the ratio of caffeine and internal standard peak areas (y) versus theoretical caffeine concentrations (x). Measurement of blood levels of lactate was performed to evaluate possible changes induced by the interventions. After collection, the blood samples were deposited in tubes with the presence of sodium fluoride. Plasma was obtained by centrifugation at 2.500 rpm at 4ºC for 20 min. The resultant plasma was stored at -20ºC until the analyses could be performed. We used commercial kits (Labtest, Brazil) and the BIO200 analyzer (Bioplus®, São Paulo, Brazil) [4]. All analyses were made in triplicate.

Statistical analysis

All values are expressed as mean ± SD and coefficient of variation (CV). The Shapiro-Wilk test was used to verify the normality of the data. Two-way analysis of variance (ANOVA) with Tukey post hoc test was used to compare the differences between the PP, PC, and CC in routine per week training, in blood measurements (caffeine and lactate), in cardiopulmonary variables (HR, VO_2, VCO_2, VE, FeO_2 and RPE), and in anaerobic power (peak power output, mean power output, fatigue index and curve power output). A Cohen's d effect size was calculated to quantify the differences between the PP, PC, and CC trials anaerobic power (peak power output, mean power output, fatigue index and curve power output) and concentration lactate. A P value of <0.05 was considered significant. Statistical analysis was performed using SPSS 16.0 software for statistical analyses (SPSS Inc., Chicago, IL, USA).

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Results

The values obtained in the maximum incremental test are shown in Table I.

Table I - *Values obtained in the maximum incremental test. Mean ± SD and coefficient of variation CV (n = 5).*

Variables	Mean ± SD	CV (%)
HR_{max}(bpm^{-1})	181 ± 8.86	4.90
VO_{2max}(ml·kg^{-1}·min^{-1})	55.12 ± 2.90	5.27
VCO_{2max}(ml·kg^{-1}·min^{-1})	67.43 ± 2.85	4.23
VE_{max}(1·min^{-1})	110.73 ± 6.94	6.27
RER_{max}	1.36 ± 0.05	4.34
Borg	10 ± 0.00	0.00
Maximum speed on treadmill (Km/h_{max})	19.64 ± 0.53	4.27

HRmax = maximum heart rate; VO_2max = maximum oxygen uptake; VCO_2max = maximum output of carbon dioxide; VEmax = maximum minute ventilation; RERmax = respiratory exchange ratio; Km/hmax = maximum speed reached. Adapted Borg rate of perceived exertion RPE (0-10 points).

In Table II, values obtained in routine per week training prior to acute experimental trials. There were no significant differences found (P>0.05).

Table II - *Training routine per week (hours). Mean ± SD (n = 5).*

	n = 5	n = 5	n = 5	
Sports Modality	PP	PC	CC	*P*
Swimming	2.2 ± 0.83	1.8 ± 0.83	1.8 ± 0.83	0.69
Cycling	4.1 ± 1.34	3.2 ± 1.64	3.4 ± 2.30	0.72
Running	3.0 ± 1.00	2.2 ± 1.30	2.8 ± 1.64	0.63

Figures 2 A, B, C, D, E and F show that between both experimental conditions (PP, PC, and CC), there were no significant differences in cardiopulmonary responses (group x time interaction: HRP=0.33; VO_2P=0.07; VCO_2P=0.08; VE P=0.08; FeO_2P=0.17) and the rate of perceived exertion (RPE P=0.51) over time for 40 min.

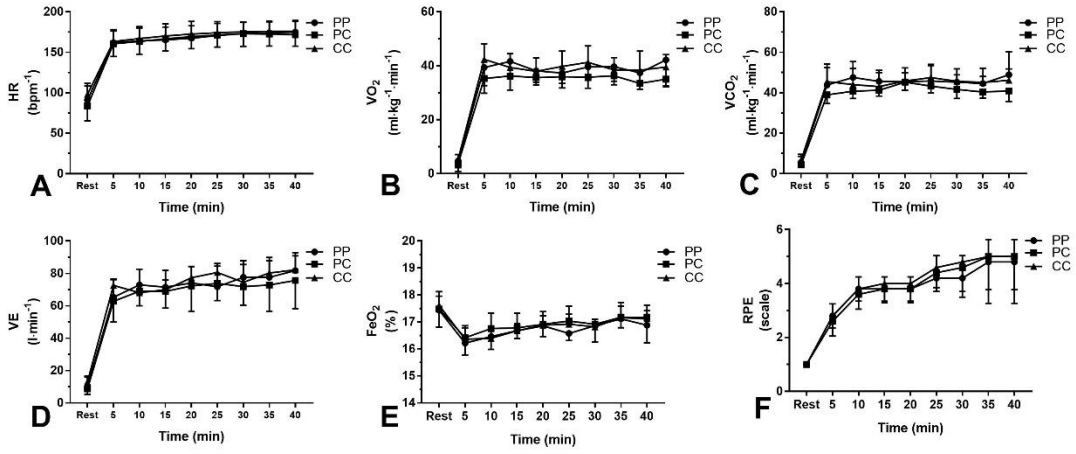

Figure 2 - *Cardiopulmonary responses of the following variables: (A) heart rate, (B) oxygen uptake, (C) output of carbon dioxide, (D) minute ventilation, (E) expired oxygen fraction, and (F) rate of perceived exertion. Error bars represent the standard deviation of the mean. (PP, placebo-placebo [n = 5]; PC, placebo-caffeine [n = 5]; CC, caffeine-caffeine [n = 5]).*

Figures 3 A, B, and C shows that between both experimental conditions, there were no significant differences in peak power output (PC, 826.99 ± 109.57 vs. PP, 779.76 ± 72.44 watts; p=0.70; CC, 822.84 ± 88.92 vs. PP,779.76 ± 72.44 watts; PC, 826.99 ± 109.57 vs. CC, 822.84 ± 88.92 watts; P=0.99), mean power output (PC,645.08 ± 74.91 vs. PP,625.89 ± 47.67 watts; P=0.98; CC,637.49 ± 67.99 vs. PP,625.89 ± 47.67 watts; P=0.97; PC, 645.08 ± 74.91 vs.CC, 637.49 ± 67.99watts; P=0.97) and fatigue index (PC, 68.61 ± 12.85 vs. PP, 75.54 ± 12.88 %;

P=0.57; CC, 79.32 ± 2.98 vs. PP, 75.54 ± 12.88 %; p=0.84; PC, 68.61 ± 12.85 vs.CC, 79.32 ± 2.98%: large effect-size d = -1.14; P=0.28). In Figure 3D, significant differences between the experimental condition in power curve in time of the 2 sec (PC, 657.05 ± 138.36 vs. PP, 497.54 ± 155.58 watts: large effect-size d = 1.08; P<0.05) and 3 sec (PC, 792.48 ± 102.31 vs. PP, 660.00 ± 119.45 watts: large effect-size d = 1.19; P<0.05) were observed.

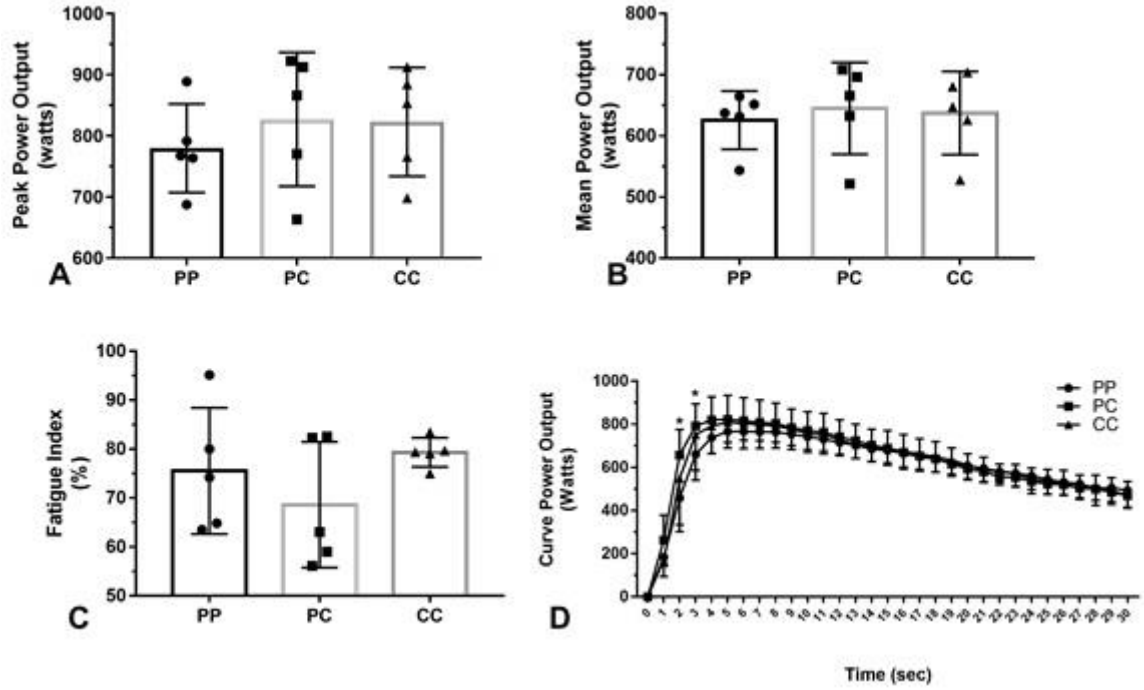

Figure 3 - *Power variables in the figures (A) peak power output, (B) mean power output, (C) fatigue index, and (D) curve power output recorded in the Wingate test of the athletes according to the experimental conditions (PP, placebo-placebo [n = 5]; PC, placebo-caffeine [n = 5]; CC, caffeine-caffeine [n = 5]). Error bars represent the standard deviation of the mean. (*) Significant difference between the PC vs. PP trials (P<0.05).*

In Figure 4, serum concentration of caffeine was not observed in the PP trial (Rest, AC, SE, WT, and R). We found significant differences (P<0.05) in serum caffeine levels in the PC trial (AC, 6.75±0.81 µg/mL-1; SE, 7.29±0.67 µg/mL-1; WT, 7.13±0.57 µg/mL-1; and R, 7.46±1.16 µg/mL-1) and CC trial (AC, 7.19±0.83 µg/mL-1; SE, 7.37±0.674 µg/mL-1; WT, 7.47±0.72 µg/mL-1; and R, 7.56±0.85 µg/mL-1) compared to PP trial. In figure 4 B, significant differences between the experimental condition PC 13.73±2.66 vs. PP 10.26±1.60 mmol.L^{-1} trials (large effect-size d = 1.58; P<0.05) in lactate blood concentration after 10-min recovery (R) were observed. There were significant differences in the concentration of lactate in PC trial (Rest 1.11±0.27 vs. ES 5.48±2.71 mmol.L^{-1}; large effect-size: d = 2.26; P<0.05) and in CC trial (Rest 1.35±0.55 vs. ES 5.59±3.79 mmol.L^{-1}; large effect-size: d = 1.56; P<0.05). There were significant differences in the concentration of lactate in PP (Rest 1.35±0.55 vs. WT 13.52±2.49 mmol.L^{-1}; large effect-size: d = 6.74; P<0.05), PC (Rest 1.11±0.27 vs. WT 14.49±2.34 mmol.L^{-1}; large effect-size: d = 8.03; P<0.05), and CC (Rest 1.35±0.55 vs. WT 14.33±3.31 mmol.L^{-1}; large effect-size: d = 5.47; P<0.05) trials.

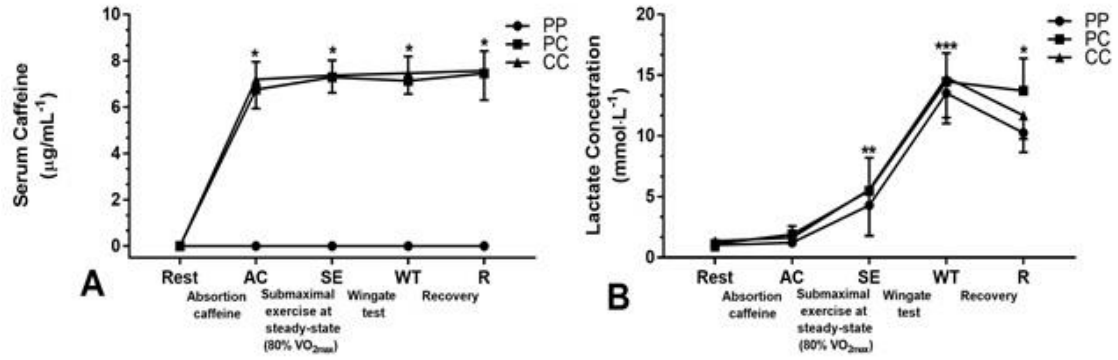

Figure 4 - *Analysis of blood samples: (A) serum caffeine, (B) lactate concentration (PP, placebo-placebo [n = 5]; PC, placebo-caffeine [n = 5]; CC, caffeine-caffeine [n = 5]). Error bars represent the standard deviation of the mean. Figure 4A: (*) Significant difference between the PC, CC vs. PP (P<0.001). Figure 4B: (*) Significant difference between the PC vs. PP in R (p<0.05). (**) Significant difference of PC and CC trials compared to Rest (P<0.01). (***) Significant difference of PP, PC, and CC trials compared with Rest (P<0.001).*

Discussion

The aim of this study was to test the hypothesis that caffeine supplementation (6 mg·kg-1 body mass) for 4 days, followed by acute intake would impact output power after performed submaximal intensity exercise. The present data observed that caffeine supplementation for 4 days, followed by acute intake did not show changes in cardiopulmonary variables (HR, VO_2, VCO_2, VE and FeO_2), in RPE and the following anaerobic power variables: peak/mean power output and fatigue index (P>0.05). However, only the PC trial compared with PP trial promoted improvements in the curve power output in 2 sec by 31.19% (P<0.05) and 3 sec by 20% (P<0.05). Additionally, a 10-min recovery was not enough to reduce blood lactate concentration in PC trials compared with PP trials (P<0.05).

Different methodologies have been used by several researchers to explain the positive ergogenic effects in the use of chronic [15] and acute caffeine [11]. The influence of a subjects' caffeine habituation is the determining factor in the verification of an ergogenic response, which is often neglected in many studies [24,13,14], although evidence shows that this interferes with physical performance after acute supplementation [15]. To minimize this conflict, all triathletes were classified as low caffeine users (242 ± 39 mg·day^{-1}) [18].

A recent positron emission tomography (PET) study Elmenhorst *et al.* [25] showed that almost half of the brain A1receptors were occupied by caffeine use when participants received an intravenous dose of 4.3 mg·kg-1 body mass, which corresponded to a plasma concentration of ~8 µg/mL-1. We used a dosage of 6 mg.kg^{-1} caffeine body mass and observed a peak serum concentration of (PC) 7.46±1.16 and (CC) 7.56±0.85 µg/mL^{-1}) (Figure 4A), similar to the study (6.59±4.44 µg/mL^{-1}) conducted by our research group, resulting in increased athletic performance [5]. This dose represents a posology of 450 mg of caffeine for 75 kg body mass (equivalent of 5 expresso cup), exceeding the usual consumption of the triathletes studied (242 ± 39 mg·day^{-1}). There is evidence that this dosage (6 mg·kg-1 body mass) may be capable of promoting reduction in the rate of perceived exertion [10]. However, we did not observe changes in RPE in PC and CC trials (Figure 2 F, P>0.05) even under a high blood lactate concentration observed after submaximal exercise in relation to Rest (Figure 4 B, P<0.01).

It is known that the rate of absorption may vary among athletes [5], and the hypothesis that 4 days habituation to caffeine could influence the increase in absorption rate was questioned [17]. However, we did not observe differences between PC and CC trials (Figure 4 A, P>0.05). The explanations are that this could facilitate the metabolization and increase the concentrations of paraxanthine, theobromine, and theophylline (caffeine metabolites not verified in the present study) [26-28]. In view of these arguments, we believe that chronic use of caffeine in the plasma is an important step in not inducing a positive ergogenic effect. Therefore, it was expected that only the acute use of caffeine (PC) could promote improvements in performance in endurance during the submaximal exercise on the treadmill [3]. However, we did not verify these changes by cardiopulmonary responses (Figures 2 A, B, C and D, P>0.05). The findings of several studies show that the main mechanism that undergoes the influence of caffeine use is the respiratory medullary complex [27,10,11]. It is known that elevated VE may increase the partial pressure of

alveolar/muscular oxygen and consequently induce improvements in performance in endurance [11]. In certain investigations, they failed to observe these findings, because the acute use of caffeine during the performance of a submaximal exercise at steady state (i.e., above 75% the VO_2max) revealed increases in diaphragm muscle fatigue [29,11]. Therefore, it does not seem prudent that fatigue of the diaphragm muscle could be associated to the findings observed in our study, because the fatigue installed in this muscle can promote significant reductions in the VE [11], and this was not observed (Figure 2 D, P>0.05). We believe that the intensity (80% VO_2max) of the submaximal exercise protocol in the present study was high about the cardiopulmonary condition of the athletes studied (Table I) to observe changes in cardiopulmonary parameters with caffeine use.

It is postulated that as a result of a resensitization of adenosine receptors (A2A/A2B) in skeletal muscle induced by the withdrawal to the chronic use of caffeine, may have induced an improvement in performance [28,17]. However, Irwin *et al.* [15] showed similar improvements in time-trial exercise with caffeine in habitual consumers regardless of a 4-day withdrawal period. Similarly, Van Soeren & Graham [24] showed equal improvements in time of exhaustion with acute caffeine supplementation in habituated consumers following no, 2-days and 4-days of caffeine withdrawal. Although our findings indicate that the acute use of caffeine (PC) improved the performance (Figure 3 D, P<0.05) and exhibition high values of lactate in recovery (PC 13.73±2.66 vs. PP 10.26±1.60 $mmol \cdot L^{-1}$ P<0.05 Figure 4 B), the 4 days period use of caffeine (CC) did not impact the mean/peak power output and fatigue index compared to placebo (PP) (Figure 3 A, B, and C P>0.05). However, the results observed individually of the 5 triathletes, PC; 4 and PC/CC; 3 triathletes responded to the use of caffeine (peak/mean power output) (Figure 3 A-B).

These results should be interpreted with caution considering inter-individual variability observed in the metabolism of caffeine. According to Ribeiro et al. [8], genetic polymorphisms in related genes to caffeine metabolism (aryl-hydrocarbon receptor [AHR], cytochrome P450 1A1 and 1A2 (CYP1A1-CYP1A2, Prenyl (Decaprenyl)) are a potential explanation for the variability in the ergogenic response to caffeine supplementation in trained athletes. Given these prior findings, it could be hypothesized that a slower metabolism would be advantageous for maximizing the ergogenic benefit of caffeine [28]. The limitation of this study is that we were unable to evaluate the polymorphisms in related genes to caffeine metabolism. This could minimize the impact on interindividual variability of a small sample of athletes and favor an improvement in statistical testing power.

Other studies [4,15,17] questioned that changes in athletes' training routines could induce doubtful findings regarding the effects of caffeine use. However, we did not observe significant changes in training routine per week (Table II, P>0.05). This suggests that triathletes have maintained similar physical capacity over the study period. Therefore, any influence on performance during the performance of the Wingate test in any experimental trial (PP, PC, and CC) is due to the 4 days period and acute supplementation.

Conclusion

In conclusion, these results indicate that caffeine supplementation (6 mg·kg-1 body mass) for 4-days, followed by acute ingestion, did not impact the triathletes output power after performed submaximal intensity exercise. The intensity (80% VO_2max) of the submaximal exercise protocol in the present study was high about the cardiopulmonary condition of the triathletes studied to observe changes in cardiopulmonary and output power parameters using the recommended caffeine dose. Thus, nutritional interventions may help athletes to adapt strategies for manipulating caffeine use. Sports and activities that alternate aerobic and anaerobic power would be applicable to the results of this study.

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