

Research article

The Effect of Acute Caffeine Intake on Resistance Training Volume, Prooxidant-Antioxidant Balance and Muscle Damage Markers Following a Session of Full-Body Resistance Exercise in Resistance-Trained Men Habituated to Caffeine

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Abstract

No previous study has analyzed the impact of caffeine intake on prooxidant-antioxidant balance and muscle damage following resistance exercise. The aim of this study was to determine the effect of 3 mg/kg of caffeine on the number of repetitions and the prooxidant-antioxidant balance and muscle damage after a session of full-body resistance exercise. Ten resistance-trained men habituated to caffeine participated in a randomized, crossover and double-blind experiment. Each participant performed two identical resistance training sessions after the intake of 3 mg/kg of caffeine or a placebo. Blood was collected before and 60 min after substance intake, just after exercise, 60 minutes after exercise, and 24 hours after testing to evaluate the activity of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, catalase), non-enzymatic antioxidants (reduced glutathione, uric acid) levels of oxidative stress markers (plasma malondialdehyde) and muscle damage markers (creatinase, lactate dehydrogenase). There were no significant differences between placebo and caffeine conditions in the total number of repetitions (180 ± 15 vs 185 ± 14 repetitions, respectively; $p = 0.276$; Effect size [ES] = 0.34), the total time under tension (757 ± 71 vs 766 ± 56 s, respectively; $p = 0.709$; ES = 0.14) or the rating of perceived exertion (13.8 ± 2.7 vs 14.7 ± 2.7 a.u., respectively; $p = 0.212$; ES = 0.32). Reduced glutathione concentration obtained 1 hour after exercise was higher with caffeine than with placebo ($p = 0.047$), without significant difference between conditions for any other prooxidant-oxidant or muscle damage marker at any time point ($p > 0.050$ for all). The oral intake of 3 mg/kg of caffeine by resistance-trained men habituated to caffeine did not enhance the number of repetitions during a medium load full-body resistance training session to failure and had a minimal impact on the prooxidant-antioxidant balance and muscle damage. The study was registered prospectively at ClinicalTrials.gov with the following ID: NCT05230303.

Key words: Antioxidant enzymes, non-enzymatic antioxidants, oxidative stress, nutrition.

Introduction

Caffeine is a commonly used psychoactive substance, consumed on regular basis by 80% of the world's population (Heckman et al., 2010). Due to its ergogenic properties, oral caffeine intake is widely used by a growing number of athletes before or during a sporting event (Del Coso et al., 2011). Indeed, several previous studies showed that caffeine, in a dose of 3-to-9 mg/kg, enhances various aspects

of exercise performance, showing benefits during endurance- (Southward et al., 2018), anaerobic- (Grgic, 2018), resistance-based exercise (Grgic et al., 2018), or in team-sports (Salinero et al., 2019) or combat sports (López-González et al., 2018). While the positive effect of acute caffeine intake on physical performance is well documented, caffeine has also been reported as a protective substance against cellular damage with significant antioxidant effects (Nikitina et al., 2015; Lee, 2000). On one hand, it may provide benefits against exercise-induced oxidative stress by scavenging reactive oxygen species (ROS) (Lee, 2000), thereby preventing or attenuating muscle damage (Merry and Ristow, 2016). Caffeine also reduces lipid peroxidation and increases the concentration and activity of antioxidant enzymes (Azam et al., 2003), and it has been suggested as a substance with potential properties to reduce inflammation after damaging exercise (Caldas et al., 2022). On the other hand, there is a growing amount of evidence suggesting that ROS may act as critical intracellular signaling molecules to promote adaptations that enhance the cellular ability to better tolerate future stress (Peternelj and Coombes, 2011). Therefore, it is possible that caffeine could interfere with ROS signaling in skeletal muscle following acute exercise and blunt favorable training adaptations, as it has been found in other supplements with antioxidant properties (Merry and Ristow, 2016).

Interestingly, only a few previous studies have analyzed the impact of acute caffeine intake on antioxidants and oxidative stress markers after exercise while the results of these investigations are conflicting (Khcharem et al., 2021; Salicio et al., 2016; Olcina et al., 2008; 2006; Tauler et al., 2013; Wang et al., 2022) and showed impact of caffeine on prooxidant-antioxidant balance (Tauler et al., 2013), but is not always a case (Khcharem et al., 2021). Moreover, the results of these studies suggest that the oxidative stress response induced by acute caffeine intake might vary depending on exercise intensity and volume. Additionally, these studies observed that both, increased exercise performance and reduced oxidative stress after exercise may concur with oral caffeine intake (Tauler et al., 2013; Wang et al., 2022). Unfortunately, all previous studies analyzed the impact of caffeine on antioxidant status following endurance exercise, and the potential interaction between caffeine supplementation and antioxidant status after high intensity resistance training is unknown.

Taking into account, that resistance exercise promotes the generation of ROS (Steinbacher and Eckl, 2015), and caffeine has potential antioxidant properties at physiological concentrations (Lee, 2000) the main aim of this study was to analyze the impact of 3 mg/kg of caffeine on the number of repetitions and selected prooxidant-antioxidant balance and muscle damage markers after high-intensity resistance full body training session in resistance-trained men habituated to caffeine. The main hypothesis was that caffeine would enhance the number of repetitions during the resistance training session and would improve the response of blood antioxidant defense after exercise.

Methods

Study design

This study was a double-blind, placebo-controlled, randomized crossover experiment, where each participant took part in two experimental sessions, acting as their own control. A member of the study team who was not involved in data collection carried out the randomization (by www.randomization.com). Thus, both, participants and researchers, were blinded to the order of the trials for each participant. Participants took part in a familiarization session with preliminary one-repetition maximum (1RM) testing on two separate experimental sessions with a one-week interval in order to achieve full recovery and substance wash-out. During the experimental sessions, participants consumed a placebo or 3 mg/kg of caffeine, administered orally 60 minutes before the onset of the exercise protocol to allow peak blood caffeine concentration at the beginning of the exercise protocol (Graham, 2001). Capsules with the appropriate dose of caffeine were used to deliver caffeine (Olimp Labs, Debica, Poland). The manufacturer also made similar placebo capsules that were packed with all-purpose flour. Sixty minutes after substance ingestion, participants performed a resistance exercise session consisting of seven exercises: a) leg press; b) sitting leg extension; c) lying leg curls; d) lat pull-down; e) chest-supported row; f) Smith machine bench press, and g) machine overhead press. In each exercise, participants performed three sets at 70% of their 1RM until volitional failure. The exercises were selected based on their common inclusion in bodybuilding- and strength-type training programs and targeted all major muscle groups of the body (Schoenfeld et al., 2022). Immediately after exercise, participants reported their rating of perceived exertion (RPE). Muscle soreness and fatigue (Overall Fatigue Scale, Overall Soreness Scale, and a Soreness on Palpation Scale (Hurley et al., 2013))

were measured prior to supplementation at baseline, immediately after exercise, and 24 hours after exercise. Blood was collected from a vein in the antecubital forearm prior to supplementation (PRE-SUPP), 60 minutes after supplementation (POST-SUPP), immediately after exercise (POST-EX), 60 minutes after exercise (+1h) and 24 hours (+24 h) after exercise (Çakır-Atabek et al., 2015). Heart rate (i.e. average and maximum; HR) during the resistance training was measured by using a heart rate monitor (Polar H10, Finland). Figure 1 contains a description of the experimental design used for the study. All testing was performed at the Strength and Power Laboratory at the Jerzy Kukuczka Academy of Physical Education in Katowice, Poland. The study protocol was approved by the Bioethics Committee for Scientific Research at the Academy of Physical Education in Katowice, Poland (03/2021), according to the ethical standards of the latest version of the Declaration of Helsinki, 2013. The study was registered prospectively at ClinicalTrials.gov with the following ID: NCT05230303.

Study participants

Fifteen healthy strength-trained male participants were recruited and volunteered after completing an ethical consent form. However, three of them did not complete all testing sessions (due to injury, illness or private reasons) and two did not donate blood 24 h after exercise in one trial. Thus, 10 participants were included in the final analysis. Data on the anthropometric and performance characteristics of the final sample of participants are depicted in Table 1. The inclusion criteria were as follows: (a) free from neuromuscular and musculoskeletal disorders, (b) advanced in resistance training with at least 3 years of experience in resistance training (participants obtained between 3 and 3.9 scores in classification by Junior et al., 2021)), (c) to have a daily caffeine consumption to be categorized as low-moderate caffeine user that implies between 25 mg and 5.99 mg/kg/day (Filip et al., 2020), measured by a validated questionnaire (Bühler et al., 2014); d) no medication or dietary supplements used within the previous month, which could potentially impact the study outcomes (i.e., beta-alanine, creatine, multi-ingredient supplements or antioxidant supplements i.e. vitamin C, D, omega-3). If a participant reported either (a) a positive nicotine status or (b) a possible caffeine allergy, they were excluded from the study. Participants maintained their regular training schedules throughout the research period and refrained from intense activity 24 hours before to testing. Participants were instructed to consume the same foods 24 hours prior

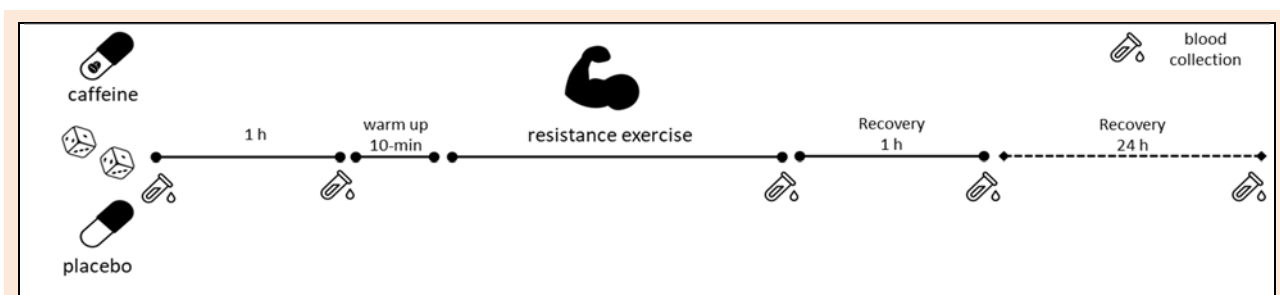


Figure 1. Study design

to each of the testing days. Also, during the study time, participants were told to follow their usual nutritional and hydration routines, including the use of caffeine-containing products as usual. However, participants were instructed to abstain from caffeine usage for 24 hours prior to each trial and for 24 hours following testing. Prior to the collection of data for each experimental session, a brief inquiry was used to confirm adherence to these guidelines.

Table 1. Participants' characteristics.

Variable [units]	(n = 10)
Age [years]	24 ± 7
Body mass [kg]	83.7 ± 8.0
Height [cm]	181 ± 6
Body fat [%]	12.9 ± 4.5
Resistance training experience [years]	6 ± 7
Leg press 1RM [kg]	218 ± 30
Sitting leg extension 1RM [kg]	104 ± 19
Lying leg curls 1RM [kg]	94 ± 17
Lat pulldown 1RM [kg]	83 ± 9
Chest supported row 1RM [kg]	55 ± 12
Bench press 1RM [kg]	81 ± 12
Overhead machine press 1RM [kg]	46 ± 8
Habitual caffeine intake [mg/kg/bm/day; mg/day]	2.2 ± 1.4; 184 ± 114

Data are presented as mean ± standard deviation; 1RM – one-repetition maximum

Familiarization session and one repetition maximum estimation

During the familiarization session, each participant was familiarized with the study procedure and the technique of performing resistance exercises. Afterward, all the participants proceeded to the standardized general warm-up, which in brief, consisted of riding on a cycling ergometer lasting approximately 5 minutes (with a resistance of ~100W and cadence of ~70rpm) and a dynamic warm-up composed of bodyweight squats, arm circles trunk rotations, side-bends, and push-ups for ten repetitions of each exercise. Then, the maximum strength evaluation in the seven exercises to standardize training intensity during the upcoming experimental session began. The exercises were carried out in the same order as in the following experimental sessions, with the same movement tempo (4/0/X/0; i.e. the eccentric phase of the exercises was performed for 4 seconds while the concentric phase was performed at the maximal possible velocity with no pause between phases) and rest intervals (3 minutes). The 1RM load for each exercise was indirectly calculated from the Epley formula (Epley, 1985). Before the maximal attempt in each exercise, participants performed 3 sets with an estimated load of 50% the estimated 1RM for 15 repetitions followed by one set at 80% the estimated 1RM for 8 repetitions. Then the load was increased to one that would allow performing a maximum of between 4 and 8 repetitions to calculate the 1RM by the Epley formula. With this load, participants were instructed to perform as many repetitions as possible, to produce a valid estimation of 1RM (Epley, 1985).

$$1RM = l \times \left(1 + \frac{r}{30}\right)$$

1 RM - one repetition maximum;

l - load;

r - number of performed repetitions

Full-body resistance exercise training session

The participants performed in two identical experimental sessions that only varied in the substance ingested. All trials were conducted between 9.00 and 12.00 in the morning to avoid the effects of the circadian rhythm on the study variables. After the warm-up procedures, which were the same as in the familiarization trial, the athletes performed a resistance exercise session consisting of seven exercises performed in the following order): a) leg press, b) sitting leg extension, c) lying leg curls, d) lat pull-down, e) chest supported row, f) Smith machine bench press, and g) overhead machine press. In each exercise, participants performed three sets (with an initial warm-up set of the given exercise of 5 repetitions performed at ~50% 1RM) at 70% of their 1RM until volitional failure using a 4/0/X/0 tempo. Rest intervals of 3 minutes were allowed between sets and exercises. We selected a training session with multi-joint exercises and 3 sets of 70%1RM since a majority of studies investigating the impact of resistance exercise-induced oxidative stress have utilized similar settings (Fisher-Wellman and Bloomer, 2009). Moreover, we decided to extend the eccentric phase duration to 4 s and perform each set until volitional failure to achieve a high time under tension (TUT) which seems to trigger higher physiological disturbance (Handford et al., 2022; Wilk et al., 2018). The TUT and the number of repetitions were assessed through video recording (Sony FDR191 AX53, Japan) for each exercise. The total TUT and number of repetitions for the whole exercise session were calculated by adding data from the seven resistance exercises.

Blood analysis

Prooxidant-antioxidant balance markers

A small part of whole blood samples was immediately assayed for reduced glutathione (GSH) by a colorimetric method (Beutler et al., 1963) with 5,5'-dithiobis-2-nitrobenzoic acid. The remaining blood was placed into test tubes to separate plasma (BD Vacutainer PPT™ Plasma Preparation Tube, UK). Plasma was obtained by centrifugation for 10 min at 1000 × g at 4° C (SIGMA 2-16KL, Sigma Laborzentrifugen GmbH, Germany). Erythrocyte sediments obtained were washed three times with cold saline (4° C). Then, blood plasma and erythrocytes were stored at -80 °C and prooxidant-antioxidant balance markers measured not later than 1 month after collection.

In the erythrocyte homogenates, it was measured activities of antioxidant enzymes, i.e. superoxide dismutase (SOD, EC 1.15.1.1), glutathione peroxidase (GPx, EC 1.11.1.9) and catalase (CAT EC 1.11.1.6). SOD activity was measured with a commercially available RANSOD 125 kit (Randox, UK). The intra- and inter-assay CV for SOD were 4.11% and 6.51%, respectively. GPx activity was measured with a commercial RANSEL RS505 kit (Randox, UK). The intra- and inter-assay CV for GPx were 5.83% and 4.03%, respectively. CAT activity was measured by the method of Aebi (Aebi, 1984). The activity of all antioxidant enzymes was measured at 37 °C and expressed per 1 g of hemoglobin (Hb) assayed using a standard cyanmethemoglobin method using a diagnostic kit (HG980, Randox, UK).

Assessment of plasma malondialdehyde (MDA) concentration as marker of lipid peroxidation was done using the thiobarbituric acid (TBARS) reaction according to Buege and Aust (Buege and Aust, 1978) by reading the absorption at $\lambda = 532$ nm using a multi-mode microplate reader (Synergy 2 SIAFRT, BioTek, USA). Standard curves were prepared using water solutions of 1,1,3,3-tetramethoxypropane (TMP) as standard.

Muscle damage markers

Plasma samples were assayed for activities of creatine kinase (CK, EC 2.7.3.2), and lactate dehydrogenase (LDH, EC 1.1.1.27), and concentrations of uric acid (UA) using diagnostic kits from Randox Laboratories (CK522, LD3818 and UA230, respectively, UK). The intra- and inter-assay CV for CK were 1.93% and 3.63%, for LDH were 2.83% and 3.38% and for UA were 0.38% and 5.64%. All biochemical tests were performed as per PN-EN ISO 9001:2015 (certificate no. PW-19912-18B) and the test manufacturers' instructions by a certified laboratory.

Side effects and assessment of blinding

A nine-item questionnaire with a yes/no answer was used to assess the prevalence of caffeine-associated side effects immediately after exercise as well as over the following 24 hours (Filip-Stachnik et al., 2021a; Pallarés et al., 2013). Additionally, participants had an opportunity to report any other side effects not included in the questionnaire in a box marked as "other side effect".

The efficiency of blinding was investigated after supplementation and after exercise by asking the participants: "Which supplement do you believe you have obtained?". They had to choose one among these three possible answers; "Caffeine", "placebo", or "I don't know".

Statistical analysis

The results were expressed as means \pm standard deviation (SD). The normality of the data for each variable was checked by the Shapiro-Wilk test. For blood markers, a two-way ANOVA (2 [substance] \times 5 [time]) for parametric data or Friedman's test for non-parametric data were employed. Post-hoc comparisons were completed using Tukey's test or Wilcoxon signed rank tests. Data on performance variables (i.e. number of repetitions, TUT, HR and RPE) was analysed using paired T-tests. Differences in side effects occurrence were determined using a Pearson's chi-squared test (χ^2). Effect sizes (ES) were calculated using Hedges *g* for repeated measures. ES of 0.00 - 0.19, 0.20 - 0.49, 0.50 - 0.79, and ≥ 0.80 represented trivial, small, moderate, and large effects, respectively. The data on participants blinding were examined using Bang's Blinding Index. All statistical analyses were conducted using SPSS (version 25.0; SPSS, Inc., Chicago, IL, USA) with the level of significance set at $p \leq 0.05$.

Results

Exercise Performance

Paired T-test found no difference in the total number of repetitions between placebo and caffeine trials (180 ± 15 vs 185 ± 14 repetitions, respectively; $p = 0.276$; $ES = 0.34$)

nor in the total time under tension (757 ± 71 vs 766 ± 56 s, respectively; $p = 0.709$; $ES = 0.14$) and RPE (13.8 ± 2.7 vs 14.7 ± 2.7 a.u., respectively; $p = 0.212$; $ES = 0.32$).

Friedman test showed a significant between-condition difference for a number of repetitions and TUT for a particular exercise ($p < 0.001$). Wilcoxon signed rank tests showed that caffeine did not modify the number of repetitions nor the time under tension in any exercise ($p > 0.05$ for all). The results of paired T-tests indicated non-significant differences between placebo and caffeine trials in mean (120 ± 15 vs 123 ± 10 bpm, respectively; $p = 0.298$), as well as peak heart rate (168 ± 12 vs 168 ± 12 bpm, respectively; $p = 0.984$) during the resistance training session.

Friedman test showed a significant between-condition difference for the results of Overall Fatigue, Overall Soreness, and Soreness on Palpation ($p < 0.001$). No significant differences between caffeine and placebo determined by Wilcoxon signed rank tests were observed for the Overall Fatigue, Overall Soreness, and Soreness on Palpation before, immediately after, and 24 h after exercise ($p > 0.05$ for all). Significant time differences were observed in reports before and immediately after and between reports before and 24 h after testing for Overall Fatigue, Overall Soreness, and Soreness on Palpation ($p > 0.05$ for all; Figure 2).

Prooxidant-antioxidant balance markers

The results of the biochemical analysis of enzymatic (SOD, CAT, GPx) and non-enzymatic antioxidant defense (GSH, UA) components are summarised in Figure 3. Friedman test showed a significant between-condition difference for GSH activity ($p < 0.001$). Wilcoxon signed-rank tests revealed that GSH activity obtained 1 hour after exercise in the caffeine condition ($Z = -1.988$, $p = 0.047$; $ES = 1.24$) was significantly increased to the corresponding time point in the placebo condition. No other significant differences in GSH activity was observed between caffeine and placebo condition at the corresponding time points. Significant differences in the placebo condition were observed between PRE-SUPP and POST-EX levels ($p = 0.007$; $ES = 1.25$), +24 h and +1 h ($p = 0.005$; $ES = 1.87$) and PRE-SUPP and +24 h levels ($p = 0.007$; $ES = 1.49$). Significant differences in the caffeine trial were observed between post-ex and +1 h levels ($p = 0.017$; $ES = 0.94$).

Two-way ANOVA showed a significant main effect of time for UA ($F = 70.455$; $p < 0.001$) and GPx ($F = 11.478$; $p < 0.001$) without main effect of substance or substance \times time interaction for UA ($p = 0.435$, $= 0.092$, respectively) and GPx ($p = 0.453$, $= 0.594$, respectively). The Tukey test showed that for UA, concentration POST-EX, +1 h, and +24 h levels were significantly increased in comparison to those obtained before exercise ($p < 0.001$; $ES = 2.17$, $= 2.50$, $= 1.29$, respectively). The activity of GPx was significantly increased +1 h in comparison to levels obtained before and immediately after exercise ($p < 0.001$; $ES = 0.88$, $= 0.95$, respectively), and significantly decreased +24 h in comparison to +1 h ($p < 0.001$; $ES = 1.08$). Two-way ANOVA showed no significant main effects of a substance, time nor substance \times time interactions for SOD ($p = 0.685$, $= 0.471$, $= 0.346$,

respectively) and MDA ($p = 0.806$, $= 0.080$, $= 0.502$, respectively). Friedman test showed no significant between-condition difference for CAT levels ($p = 0.700$). Figure 3 summarized prooxidant-antioxidant balance markers.

Muscle damage markers

Two-way ANOVA showed a significant main effect of time for LDH ($F = 16.416$; $p < 0.001$), without main effect of substance or substance \times time interaction ($p = 0.102$, $= 0.379$, respectively). The Tukey test showed that LDH levels POST-EX, +1 h, +24 h were significantly increased in comparison to PRE-SUPP ($p < 0.05$; $ES = 1.98$, $= 1.44$, $= 0.51$, respectively). Friedman test showed a significant between-condition difference for CK levels ($p < 0.001$). In the placebo condition, CK levels obtained POST-EX, +1h, +24 h were significantly increased in comparison to PRE-SUPP ($p = 0.005$; $ES = 0.81$, $= 1.01$, $= 0.80$, respectively). In the caffeine condition, CK values obtained POST-EX, +1h, +24h were significantly increased in comparison to PRE-SUPP ($p = 0.005$; $ES = 0.98$, $= 1.30$, $= 1.33$,

respectively), and increased between +1 h and POST-EX ($p = 0.037$; $ES = 0.55$). Figure 4 summarized plasma levels of muscle damage markers.

Side effects and assessment of blinding

After the caffeine trial, participants reported increased urine output (10%), headache (20%), increased vigor/activeness (20%), gastrointestinal problems (10%) and perception of performance improvement (20%) and increased mental stress (10%). After the placebo condition, participants indicated headache (20%), gastrointestinal problems (20%), and tiredness (10%). The prevalence of side effects was similar between caffeine and placebo conditions ($p > 0.05$ for all). In the pre-exercise evaluation, 60% and 30% of participants, respectively, correctly recognized the caffeine and placebo conditions (Bang Index = 0.00 and 0.60, respectively). In the post-exercise examination, 60% and 50% of participants, respectively, correctly recognized the caffeine and placebo conditions (Bang Index = 0.00 and 0.40, respectively).

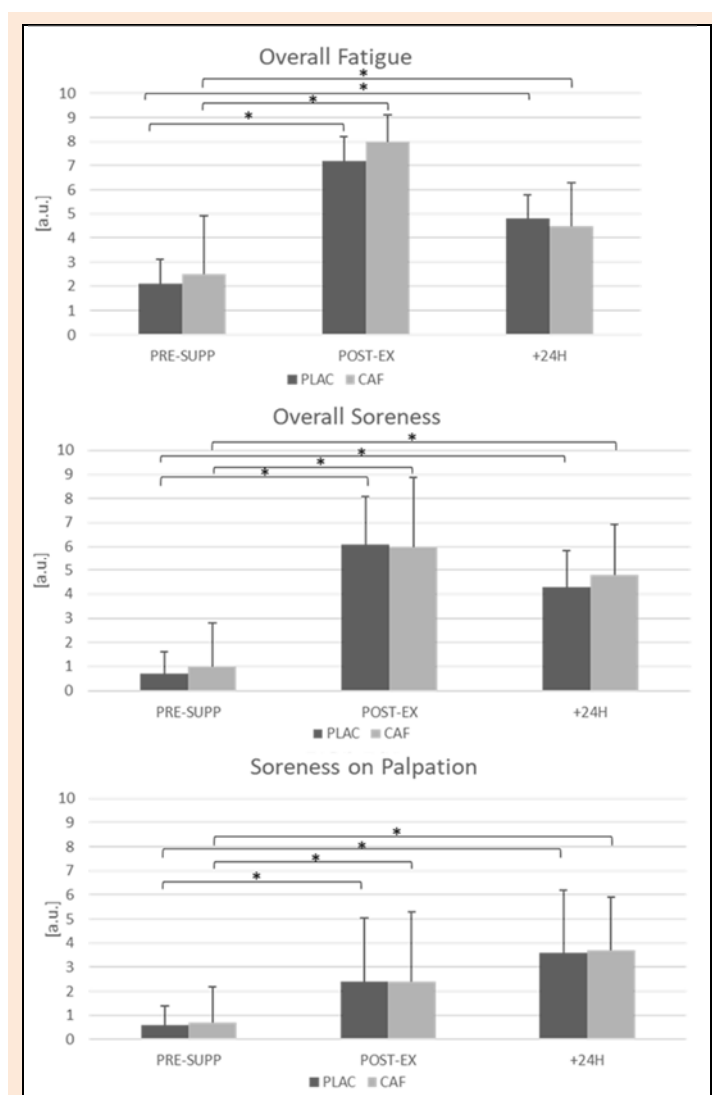


Figure 2. Time course of Overall Fatigue, Overall Soreness, and Soreness on Palpation before and after a full-body resistance training session with oral caffeine intake (CAF) or placebo (PLA) in resistance-trained men habituated to caffeine. Data are presented as mean \pm standard deviation. PRE-SUPP – prior to supplementation; POST-EX – immediately after exercise; +24 h – 24 hours after exercise. *significant difference between time points ($p < 0.050$).

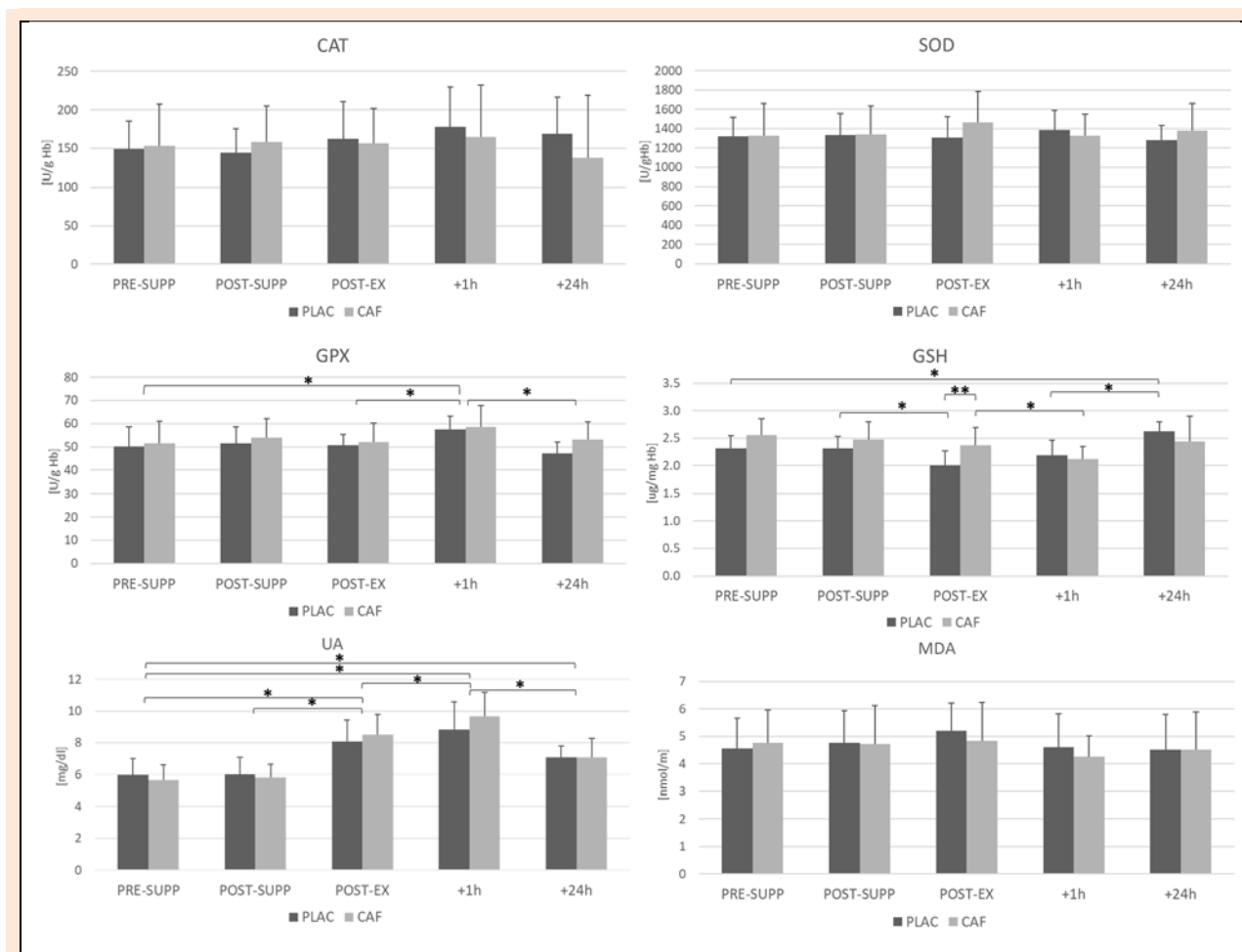


Figure 3. Time course of blood markers associated with pro-oxidant antioxidant balance before and after a full-body resistance training session with oral caffeine intake (CAF) or placebo (PLA) in resistance-trained men habituated to caffeine. Data are presented as mean \pm standard deviation. CAT – catalase; SOD – superoxide dismutase; GPx – glutathione peroxidase; GSH – reduced glutathione; UA – uric acid; MDA – malondialdehyde; PRE-SUPP – prior to supplementation; POST-SUPP – 60 minutes after supplementation; POST-EX – immediately after exercise; +1h – 60 minutes after exercise; +24h – 24 hours after exercise. *significant difference between timepoints ($p < 0.050$); ** significant difference between caffeine and placebo ($p < 0.050$).

Discussion

The present study investigated the effects of 3 mg/kg of caffeine on the number of repetitions, antioxidant status, oxidative stress, and muscle damage markers after a high-intensity full-body resistance training session. The main finding of this study is that, despite there were no differences in resistance training volume between conditions, GSH concentration obtained immediately after exercise was significantly increased in the caffeine condition in comparison to corresponding value in the placebo condition. However, no other differences between caffeine and placebo conditions were observed in enzymatic antioxidants (SOD, CAT, GPx) as well as non-enzymatic antioxidants (UA). Oral caffeine intake did not modify the time course of recovery of lipid peroxidation products (MDA) and muscle damage markers (CK and LDH) after exercise. Collectively, all these outcomes suggest that the oral intake of 3 mg/kg of caffeine did not enhance the number of repetitions during a full-body resistance training session and had minimal impact on the prooxidant-antioxidant balance and muscle damage induced by the exercise. Our results revealed that 3 mg/kg

of caffeine consumed before a full body resistance training session has a significant impact on GSH concentration obtained immediately after exercise (with a large ES = 1.24), at least in comparison to the same exercise training session with placebo intake. Since GSH is an important antioxidant that can remove ROS and is considered a biomarker of redox imbalance at the cellular level (Marrocco et al., 2017), a higher level of GSH after exercise with caffeine suggests reduced oxidative stress. In other words, as there were no differences in the number of repetitions and the time under tension, it is possible that the production of ROS was similar between conditions; hence, the lower GSH with caffeine was likely used to neutralize more ROS than in the placebo condition. The possible differences between conditions can be explained by the antioxidant properties of caffeine, as reported previously (Nikitina et al., 2015; Lee, 2000). Indeed, previous studies suggested that caffeine increased GSH concentration in humans (Metro et al., 2017), as well as in animal models (Aoyama et al., 2011), and consequently may help to resist oxidative stress. In the current study, the antioxidant properties of caffeine after exercise were partly present in absence of any ergogenic benefit of caffeine.

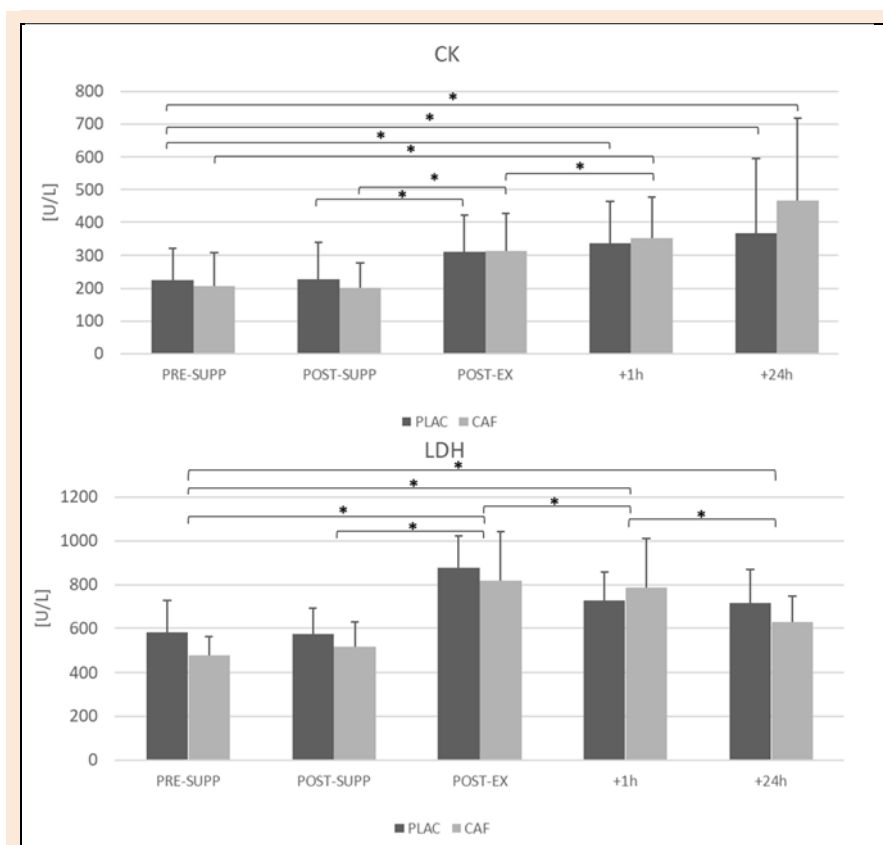


Figure 4. Time course of blood markers associated to muscle damage before and after a full-body resistance training session with oral caffeine intake (CAF) or placebo (PLA) in resistance-trained men habituated to caffeine. Data are presented as mean \pm standard deviation. CK - creatine kinase; LDH - lactate dehydrogenase; PRE-SUPP - prior to supplementation; POST-SUPP - 60 minutes after supplementation; POST-EX - immediately after exercise; +1h - 60 minutes after exercise; +24 h - 24 hours after exercise. *significant difference between timepoints ($p < 0.050$).

Further experiments should determine if this potential benefit of caffeine for resistance-based exercise is also present when caffeine increases exercise performance. Data obtained with endurance exercise protocols have found that both, increased exercise performance and reduced oxidative stress after exercise are present with oral caffeine intake (Tauler et al., 2013; Wang et al., 2022), but this should be confirmed for resistance exercise.

However, the results of the current investigation are contrary to other previous studies where the impact of caffeine on post-exercise GSH concentration was not observed (Khcharem et al., 2021; Wang et al., 2022). Although the direct comparison is difficult due to differences in exercise protocol, it is worth noting that those studies analyze the impact of caffeine on endurance type of exercise (i.e., triathlon (Wang et al., 2022) and 3-km run (Khcharem et al., 2021)). Moreover Khcharem et al. (2021) observed a significant increase in post-exercise GSH concentration (in range +9 % - +14% in both conditions), while results of the current investigation showed significantly decreased concentrations after exercise (in range of -7% - -13% for both conditions). Interestingly, the observation of the animal model reveals that the response of GSH to exercise varies depending on muscle fiber type involved in the exercise (Ji et al., 1992; Powers et al., 1999). Specifically, type I muscle fibers (slow-twitch oxidative), contains six-fold higher GSH content (~ 3 mM) than the type IIb muscle fibers (fast-

twitch glycolytic) and adaptation may be related to the rate of GSH utilization versus the capacity of GSH uptake within each fiber type (Powers et al., 1999). Thus, it cannot be excluded, that impact of caffeine intake on GSH concentration might be dependent on the type of exercise and/or fiber type.

The results of the present study showed no effect of caffeine on muscle damage markers after exercise in comparison to the placebo, which is in line with several previous studies that found no impact of caffeine on LDH (Hurley et al., 2013; Machado et al., 2010; Ribeiro et al., 2020; Vimercatti et al., 2008) and CK activities (Hurley et al., 2013; Kazman et al., 2020; Machado et al., 2010; Ribeiro et al., 2020; Vimercatti et al., 2008). Although the design of the current study seems to be more exhaustive than regular resistance training as it involved a high training volume of mainly multi-joint exercises and multiple sets performed until volitional failure, the protocol is comparable to previous studies that also used resistance-trained participants (Hurley et al., 2013 and Machado et al., 2010). Interestingly, despite this type of exercise, the levels of muscle damage after exercise and 24 h after were modest which could have reduced the potentially benefits derived from caffeine intake. Therefore, further studies should investigate the properties of caffeine supplementation in other forms of resistance exercise, as in velocity-based training that do not imply

exercise until failure but especially in protocols that produce higher levels of muscle damage.

Despite the fact that it has been postulated that caffeine use would disrupt adenosine receptor signaling and enhance the acute inflammatory response, which could lead to muscle damage (Bassini-Cameron et al., 2007; Hatfield et al., 2009), in the current study only significant differences between time points were observed. The lack of changes between conditions in the current study might be explained by no significant differences in resistance training volume when participants ingested caffeine and placebo. Similarly, participants' reports of RPE and muscle soreness were not different between placebo and caffeine conditions. Because the total number of repetitions and TUT in both conditions were similar, it can be assumed that CK and LDH activities may be related to the total work performed during the resistance exercise sessions and not to the direct effect of the substances ingested.

The results of the current study showed no significant differences in resistance training volume explained in the number of repetitions, as well as in TUT between 3 mg/kg of caffeine and placebo. Although a plethora of research analyzed the impact of caffeine intake on resistance performance, data regarding real-life resistance training sessions are scarce (Astorino et al., 2011; Da Silva et al., 2015; Duncan et al., 2012; Filip-Stachnik et al., 2021b; Giráldez-Costas et al., 2020; Green et al., 2007; Polito et al., 2019; Salatto et al., 2020). Interestingly, previous research found a positive effect of caffeine on the number of repetitions performed to failure during multiple-set resistance exercise workouts (Da Silva et al., 2015; Green et al., 2007; Polito et al., 2019; Salatto et al., 2020), although this was not always the case (Astorino et al., 2011). The possible explanation of differences between these studies with ergogenic properties of caffeine during resistance exercise sessions and the current study might be associated with the caffeine dose, the training protocol or the type of participants used. The total amount of work performed during the resistance training sessions of the current study (7 exercises x 3 set to failure) is higher in comparison to previous investigations (2-3 exercise x 3 sets to failure (Da Silva et al., 2015; Green et al., 2007; Polito et al., 2019; Salatto et al., 2020)), where a positive effect of caffeine was observed. Moreover, the exercise protocol of the current investigation included accentuation of the eccentric phase for 4 seconds, which resulted in possible higher effort in comparison to previous investigations (2 s for each concentric and eccentric phase or not defined). Interestingly, Pallarés et al. (2013) observed that a small caffeine dose is enough to improve high-velocity muscle actions against low loads, whereas a higher caffeine dose is necessary against high loads. Similarly, in some previous investigations with a positive impact of caffeine on performance during a resistance training session, participants ingested high (~9 mg/kg) (Filip-Stachnik et al., 2021a; Salatto et al., 2020) or moderate (6 mg/kg) (Da Silva et al., 2015; Green et al., 2007; Polito et al., 2019) caffeine doses, which is higher than 3 mg/kg of caffeine provided into the current study. Thus, it is possible, that the at least a moderate dose of caffeine is needed to when the resistance training extends

involves more exercises and sets. Last, it is worth noting that the participants involved in the current study had an average daily caffeine intake of 2.2 mg/kg/day (183 mg/day) and they would likely present some tolerance to the ergogenic benefits of oral caffeine intake (Lara et al., 2019). Interestingly, the diminished effect of caffeine ingestion in the current study may be related to the dose employed as it was slightly above their daily caffeine intake (3.0 vs 2.2 mg/kg). Recently, Filip-Stachnik et al. (2021b) observed that 6 mg/kg of caffeine were needed to enhance performance during a bench press exercise routine in participants habituated to caffeine (~1.56 mg/kg/day). These authors suggested that caffeine enhanced power output during the resistance training session because the dose of caffeine was ~4-fold than participant's habitual caffeine intake. The participants involved in the current study ingested a dose which was only ~1.5 times higher than their daily level of habitual caffeine intake. Thus, it is possible that caffeine users might need a dose of caffeine several times higher than their daily caffeine intake before high-intensity resistance training sessions to obtain meaningful improvements, particularly to overcome the tolerance developed by their chronic caffeine ingestion.

In addition to its strengths, the current study contains several limitations which should be addressed: (1) the study did not include an analysis of caffeine concentration in either blood or urine samples to assess peak caffeine concentration and the time course of caffeine wash-out (2) there was no genetic testing for polymorphism associated with caffeine (3); the study was conducted only on young, trained men; therefore, the findings may not be generalizable to women and inactive people; 4) although the performed resistance training was similar in the case of intensity and volume to the typical workout for resistance-trained individuals it has to be emphasized that performing each set to volitional failure and extending the eccentric phase is not common. Thus, caffeine's potential benefits on the antioxidant-prooxidant status should be studied with less demanding forms of resistance exercise. Finally, we analyzed only the effects of caffeine intake in mild caffeine consumers, and the generalizability of these results to the population with other levels of caffeine consumption remains unclear. Specifically, the study of caffeine benefits on performance and the prooxidant-antioxidant balance and muscle damage after resistance exercise should be performed in caffeine-naïve individuals, as the lack of habituation to caffeine may potentiate caffeine-induced effect during and after exercise (Lara et al., 2019).

Conclusion

The main finding of this study is that despite no differences in resistance training volume between conditions, GSH concentration obtained immediately after resistance exercise in the caffeine condition was significantly increased in comparison to corresponding values in the placebo condition. However, no other significant differences between caffeine and placebo were observed in enzymatic (SOD, CAT, GPx) and non-enzymatic antioxidant defense (UA) and a marker of lipid peroxidation (MDA) as well as

muscle damage markers (CK and LDH). Additionally, caffeine has no impact on RPE and muscle soreness. Collectively, these outcomes suggest that the oral intake of 3 mg/kg of caffeine by resistance-trained men habituated to caffeine did not enhance the number of repetitions during a medium load full-body resistance training session to failure and had a minimal impact on the prooxidant-antioxidant balance and muscle damage.

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Key points

- 3 mg/kg of caffeine did not enhance the number of repetitions during a full-body resistance training session in resistance-trained men habituated to caffeine.
- 3 mg/kg of caffeine had minimal impact on the prooxidant-antioxidant balance and muscle damage induced by the exercise.
- It is possible that caffeine may provide some benefits to reduce oxidative stress after a resistance exercise training session. Further experiments should investigate if these reduced oxidative stress with caffeine counteract some of the training adaptations induced by resistance exercise (f.e. muscle hypertrophy).

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