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Single and combined effect of beetroot juice and caffeine intake on muscular strength, power and endurance performance in resistance-trained males

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To examine the single and combined effect of acute beetroot juice and caffeine supplementation in muscular strength, power, and endurance performance. Thirteen resistance-trained males participated in a triple-blind, cross-over, randomized controlled-trial with four conditions: (a) caffeine (CAF); (b) beetroot juice (BJ); (c) caffeine and beetroot juice (CAF + BJ); (d) placebo (PLA). Participants ingested 70 mL of beetroot juice, concentrated NO_3^--rich beverage (BJ, 6.4 mmol NO_3^-) or PLA (~ 0.04 mmol NO_3^-) 180 min and caffeine or placebo (3 mg/kg) 60 min before the trial. Muscular strength/power was evaluated at 25%, 50%, 75%, 90% and 100%1RM and muscular endurance at 65%1RM, in bench press (BP) and back squat (BS). In all tests, mean (V_{mean} and W_{mean}) and peak (V_{peak} and W_{peak}) velocity and power output were measured. In BS, muscular strength/power showed a supplement-by-load effect in V_{mean} and W_{mean} ($P < 0.05$, $\eta_p^2 = 0.167–0.173$), with caffeine increased compared to placebo at 75%, 90% and 100%1RM (9–25%, $P < 0.005$, $g = 0.51–1.47$); while in muscular endurance, significant differences were found in number of repetitions, V_{mean} and W_{mean} ($P < 0.05$, $\eta_p^2 > 0.277$), in all experimental groups (CAF, BJ and CAF + BJ) compared to placebo (6–17%, $P < 0.05$, $g = 0.46–94$). No differences in muscular strength/power or endurance were found in BP. Single and combined acute beetroot juice and caffeine intake increased muscular endurance performance at 65%1RM in back squat but not in bench press exercise.

Keywords Dietary supplements, Caffeine, Nitrates, Sports performance, Resistance exercise

Caffeine is a dietary supplement with strong scientific evidence for enhancing short-duration efforts performance[1], and a high prevalence of use among competitive athletes (~ 75% of Olympic athletes)[2]. Nitrate (NO_3^-) has also been proposed as an ergogenic supplement in short-duration efforts[3], but its prevalence is low among the athletic population[4]. Since NO_3^- and caffeine could enhance performance through different physiological mechanisms[1,5], the ingestion of both supplements could stimulate an additive ergogenic effect. However, the interaction between NO_3^- and caffeine has been limited study, particularly in muscular strength, power, and endurance performance, despite being a critical performance component in several sports.

Nitrate (NO_3^-) intake increases nitric oxide bioavailability via the NO_3-nitrite-NO pathway, which allows for the improvement of skeletal muscle function via type II muscle fibers[5,6] and by reducing ATP cost of muscle force production[7,8]. Moreover, nitrate may improve local perfusion, fatigue resistance, and contractility of low-oxygenated type II (fast twitch) muscle fibers[9] as well as increase neurotransmitter release[10] and attenuate muscle K+ efflux[11] that could benefit neuromuscular performance. Caffeine's effect mainly occurs in the central nervous system (CNS) by antagonizing adenosine receptors, reducing fatigue and perceived effort, increasing alertness and vigour, and facilitating muscle fiber recruitment during muscle contraction[1]. Besides, caffeine may promote

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intracellular calcium ion (Ca^{2+}) mobilization, stimulating force production[12] and delaying fatigue caused by a gradual reduction in Ca^{2+} bioavailability[13]. Although both supplements may enhance force production and delay fatigue, the combined effect of both supplements has been scarcely explored.

The isolated ergogenic effect of NO_3^- and caffeine occurs through different mechanisms of action, which may indicate that the combined ingestion of both supplements could produce a synergic effect. This idea has been previously explored in 20-km cycling time trial performance[14], in time-to-exhaustion at 80% VO_{2peak} after 30 min submaximal cycling test[15], in cycling time trial simulating the 2012 London Olympic Games course (29.35 km females and 43.83 km males)[16] or in 2 × 5 min submaximal running bout (70 and 80% VO_{max}) and 1-km time trial[17]. In these studies, caffeine improved performance in the time trial, but no isolated or combined effect of nitrate was found in any of them. Nevertheless, to our knowledge, no studies have explored the potential additive effect of these two supplements in short duration effort (e.g., ≤ 30 s) despite isolated intake of NO_3^-[3,18] and caffeine[19,20] improving muscular strength and power production. Therefore, this study aimed to examine the single and combined effect of acute beetroot juice and caffeine supplementation on muscular strength, power and endurance performance in resistance-trained male participants.

Results

Muscular strength and power

Differences in mean, peak and time to reach peak velocity and power output in the bench press exercise are illustrated in Fig. 1. No differences in supplement effect or supplement by load effect was found in mean velocity (V_{mean}, $P = 0.269–0.321$, $\eta_p^2 = 0.113–0.099$), peak velocity (V_{peak}, $P = 0.135–0.621$, $\eta_p^2 = 0.164–0.070$), time to reach V_{peak} ($P = 0.264–0.496$, $\eta_p^2 = 0.114–0.063$), mean power output (W_{mean}, $P = 0.253–0.188$, $\eta_p^2 = 0.118–0.131$), peak power output (W_{peak}, $P = 0.354–0.323$, $\eta_p^2 = 0.083–0.093$) or time to reach W_{peak} ($P = 0.263–0.498$, $\eta_p^2 = 0.114–0.062$) in the bench press exercise.

Differences in mean, peak and time to reach peak velocity and power output in the back squat exercise are illustrated in Fig. 2. Supplement effect was found in V_{mean}, ($P = 0.032$, $\eta_p^2 = 0.234$) while supplement by load effect was found in V_{mean}, ($P = 0.031$, $\eta_p^2 = 0.173$) and W_{mean} ($P = 0.042$, $\eta_p^2 = 0.167$). In V_{mean}, the differences were found when caffeine was compared to placebo at 75%1RM (0.447 ± 0.058 vs 0.395 ± 0.059 m/s; $P = 0.008$, $g = 0.88$), at 90%1RM (0.319 ± 0.042 vs 0.247 ± 0.050 m/s; $P = 0.002$, $g = 1.47$) and 100%1RM (0.233 ± 0.045 vs 0.173 ± 0.039 m/s; $P = 0.002$, $g = 1.34$). In W_{mean}, the differences were found when caffeine was compared to placebo at 75%1RM (798 ± 124 vs 699 ± 100 W; $P = 0.008$, $g = 0.82$), at 90%1RM (552 ± 107 vs 501 ± 119 W; $P = 0.041$, $g = 0.52$) and 100%1RM (448 ± 111 vs 370 ± 99 W; $P = 0.009$, $g = 0.70$).

Muscular endurance

Differences in the number of repetitions, mean velocity, and power output in the bench press and back squat exercises are illustrated in Figs. 3 and 4, repectively. In the bench press, no statistically significant differences were found in the number of repetitions, V_{mean}, V_{peak}, time to reach V_{peak}, W_{mean}, W_{peak} or time to reach W_{peak} ($P = 0.090–0.251$, $\eta_p^2 = 0.115–0.212$). In the back squat exercise, no differences were found in V_{peak}, time to reach V_{peak}, W_{peak} or time to reach W_{peak} ($P = 0.062–0.111$, $\eta_p^2 = 0.246–0.403$), but in contrast, statistically significant differences were found in the number of repetitions performed ($P = 0.030$, $\eta_p^2 = 0.277$), V_{mean} ($P < 0.001$, $\eta_p^2 = 0.470$) and W_{mean} ($P = 0.001$, $\eta_p^2 = 0.458$). In the number of repetitions, CAF (20 ± 4 reps; $P = 0.043$, $g = 0.94$), BJ (19 ± 4; $P = 0.049$, $g = 0.57$) and CAF + BJ (19 ± 4; $P = 0.022$, $g = 0.50$) increased performance compared to placebo (17 ± 4 reps). In V_{mean}, CAF (0.361 ± 0.045 m/s; $P = 0.008$, $g = 0.75$), BJ (0.350 ± 0.041 m/s; $P = 0.008$, $g = 0.54$) and CAF + BJ (0.366 ± 0.034; $P = 0.001$, $g = 0.92$) increased performance compared to placebo (0.328 ± 0.036 m/s). In W_{mean}, CAF (574 ± 80 W; $P = 0.001$, $g = 0.55$), BJ (570 ± 91; $P = 0.019$, $g = 0.46$) and CAF + BJ (588 ± 93; $P < 0.001$, $g = 0.67$) increased performance compared to placebo (528 ± 73 W).

Isometric strength and vertical jump test

No differences were found in isometric handgrip strength in the dominant hand ($P = 0.511$, $\eta_p^2 = 0.047$) and the non-dominant hand ($P = 0.422$, $\eta_p^2 = 0.060$), the isometric mid-thigh pull test ($P = 0.148$, $\eta_p^2 = 0.160$), CMJ height ($P = 0.171$, $\eta_p^2 = 0.139$) or CMJ power ($P = 0.052$, $\eta_p^2 = 0.231$).

Questionnaires and scales

Caffeine intake stimulated a statistically significant reduction in fatigue perception in CAF and BJ + CAF compared to PLA (2.15 ± 1.1 & 2.00 ± 0.1.1 vs 2.67 ± 0.80; $P < 0.005$, $g = 0.32$). No statistical differences in side effects were found in mood state, nervousness, activeness, insomnia, gastrointestinal discomfort, headache and irritability. Finally, 15% (2 of 13) of participants correctly guessed the order of the supplement ingested, a 23% (3 of 13), 15% (2 of 13), 30% (4 of 13) and 30% (4 of 13) guessed the ingestion of PLA, BJ, CAF and BJ + CAF, respectively.

Discussion

The purpose of this study was to evaluate the single and combined effect of acute beetroot juice and caffeine supplementation on muscular strength, power and endurance performance in resistance-trained male participants. Our results suggest that when muscular strength and power are required, only the isolated ingestion of caffeine improves performance by increasing mean velocity and power production at 75%, 90%, and 100% 1RM. However, when muscular endurance is required, the single and combined ingestion of beetroot juice and caffeine improves performance in the back squat but not in the bench press exercise, by increasing the number of repetitions, mean velocity, and power production. This effect was more pronounced in mean velocity and power production when both supplements were combined suggesting a potential summative effect.

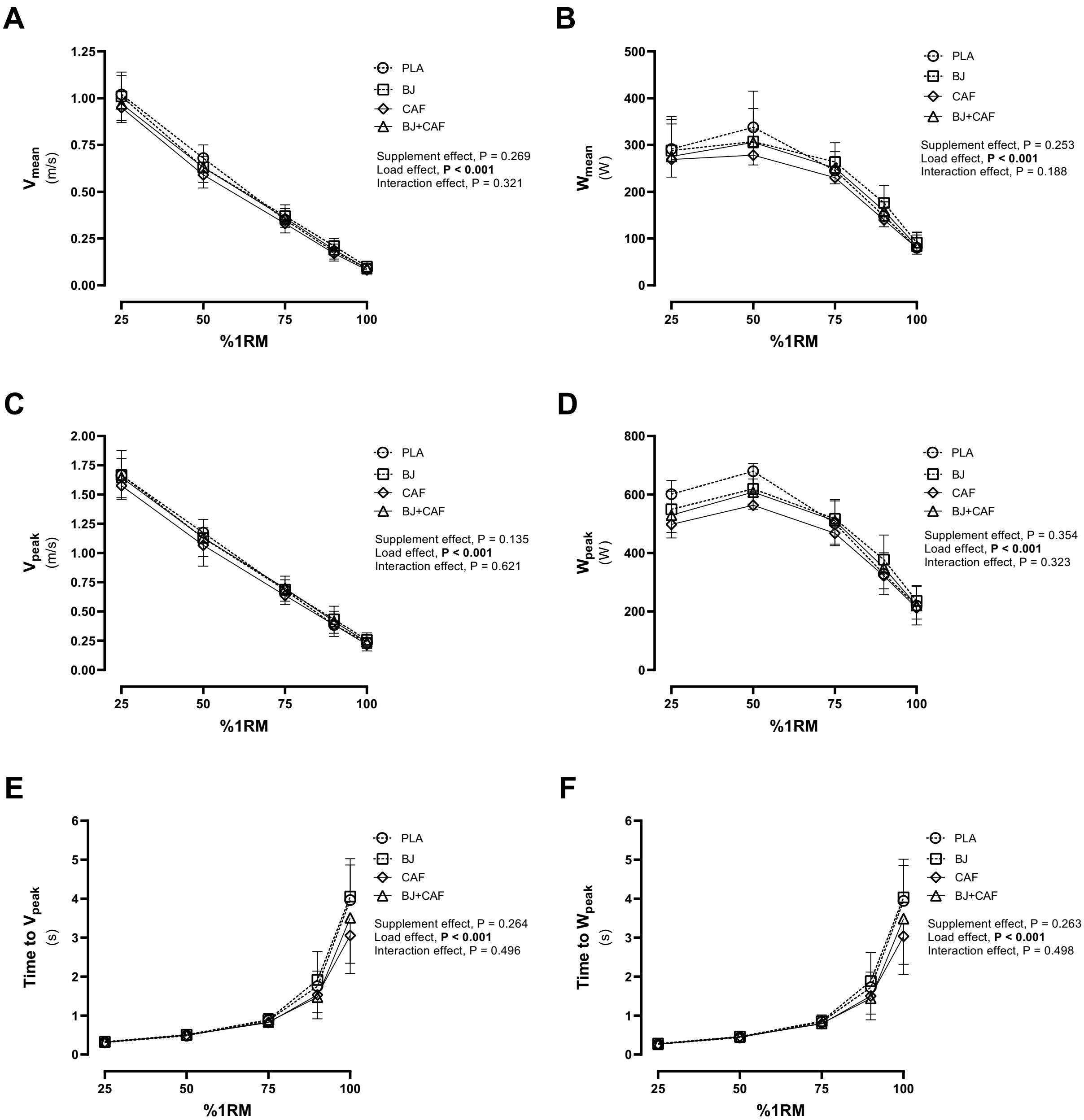

Fig. 1. Muscular strength and power tests differences in mean, peak and time to reach peak velocity and power production among experimental conditions in the bench press exercise. Mean velocity (V_{mean}, **A**), mean power (W_{mean}, **B**), peak velocity (V_{peak}, **C**), peak power (W_{peak}, **D**), time to reach V_{peak} (**E**) and time to reach W_{peak} (**F**). *PLA* placebo, *BJ* beetroot juice, *CAF* caffeine, *BJ + CAF* beetroot juice and caffeine.

Previous research has reported that acute caffeine intake improves V_{mean}, V_{peak}, W_{mean} and in muscular strength, power and endurance[21–23]. In muscular strength and power, different doses of caffeine, from 3 to 9 mg/kg of body mass, promote an increase in velocity and power at 25%, 50%, 75% and 90% of 1RM[24,25]. After low-dose of caffeine intake (3 mg/kg), Pallares et al.[25] found an increase in mean propulsive velocity at 25% and 50% 1RM in bench press and full squat exercise, and at 75% 1RM for full squat; Mora-Rodriguez[26] observed this effect for full squat but not in bench press exercise; while Ruiz-Fernandez et al.[19] and Montalvo-Alonso et al.[27] found an increase in V_{mean} and W_{mean} in back squat, but not in bench press exercise, at moderate-to-high loads ≥75% of 1RM. Our results are in line with this evidence since caffeine increases V_{mean} and W_{mean} at 75%, 90% and 100% 1RM in the back squat but not in the bench press exercise.

Beetroot juice supplementation has been reported to improve maximal muscular strength production[28] mainly by promoting type II muscle fiber recruitment[6]. Some evidence has questioned this idea based on the lack of differences found in maximal strength and power of the upper leg in voluntary isometric and isokinetic contractions[29]. However, in isotonic exercises (e.g., resistance exercise), there is evidence that NO_3^- supplementation can improve performance[30,31]. Nevertheless, when a pause was introduced between the

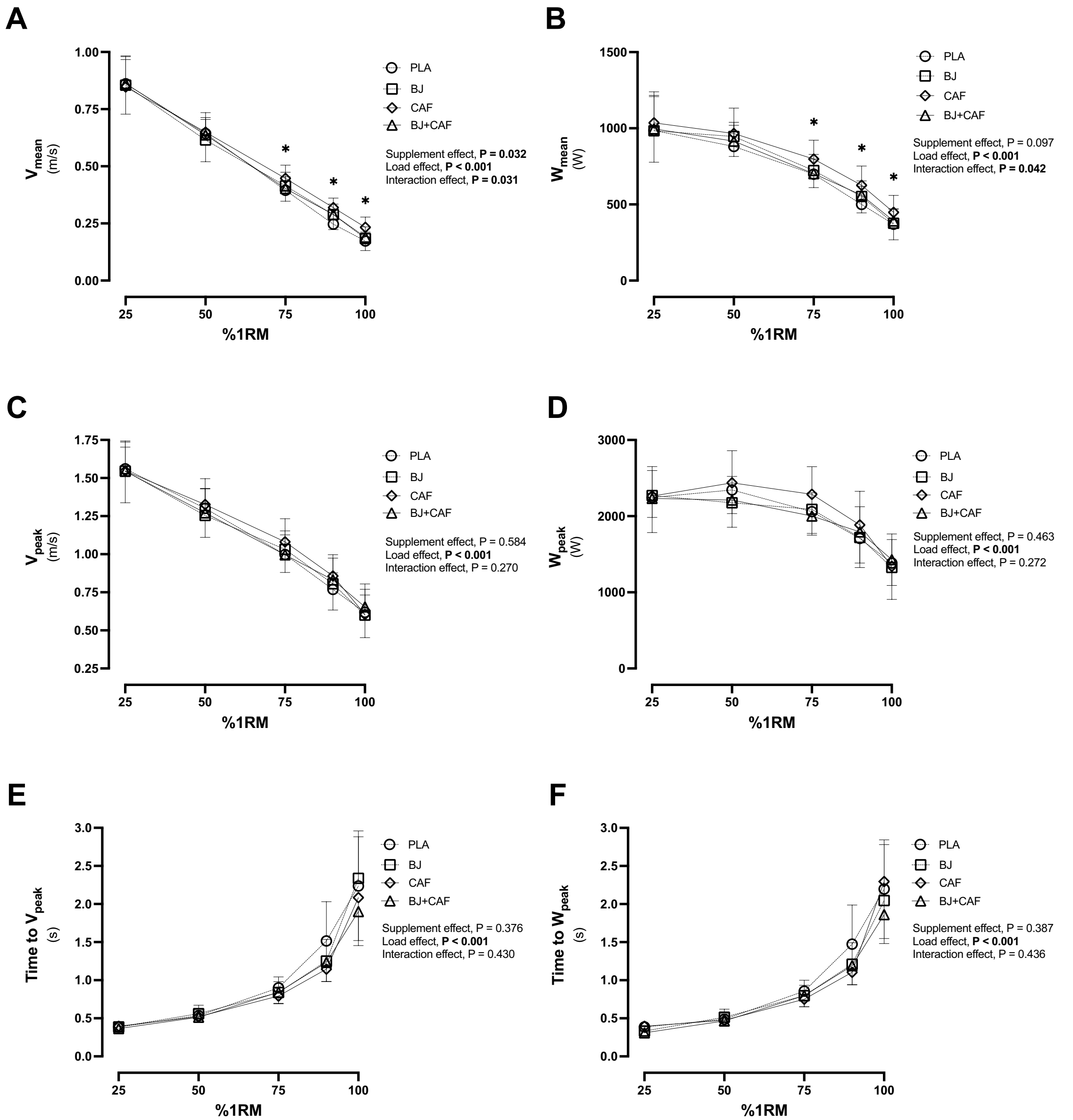

Fig. 2. Muscular strength and power tests differences in mean, peak and time to reach peak velocity and power production among experimental conditions in the back squat exercise. Mean velocity (V_{mean}, **A**), mean power (W_{mean}, **B**), peak velocity (V_{peak}, **C**), peak power (W_{peak}, **D**), time to reach V_{peak} (**E**) and time to reach W_{peak} (**F**). *P < 0.05 CAF compared to PLA. *PLA* placebo, *BJ* beetroot juice, *CAF* caffeine, *BJ + CAF* beetroot juice and caffeine.

eccentric and concentric phases of each repetition, to improve test reproducibility, no ergogenic effect of NO_3^- was detected[30]. In line with this, in our study, BJ did not cause an improvement in muscular strength and power performance in bench press or back squat exercises in any of the %1RM evaluated (25%, 50%, 75%, 90% and 100%1RM), in isometric contractions or in countermovement jump (CMJ) height or power output. This could be explained because NO_3^- supplementation has been demonstrated to increase velocity and power in eccentric contractions[32] and the enhancement in muscle force is specific in isokinetic contractions to very high angular velocities[33]. The specific effect of beetroot juice on the type II muscle fibers[4], the speed of contractions[33] and contributions of the eccentric contraction strength[32] could be affected by the procedure selected in this study. Therefore, the pause between the eccentric and concentric phases could be a potential factor that blunts the ergogenic effect of beetroot juice.

Muscular endurance refers to skeletal muscles' ability to resist fatigue by maintaining or delaying the diminution of force and power production during repeated muscle contraction. Previous systematic reviews and meta-analyses indicate that caffeine can improve muscular endurance by a 6–7%[21,34,35], mainly due to an increase in the number of repetitions performed per set after the acute intake of this substance[36–39]. Unfortunately, there is scarce evidence evaluating multiple performance variables during muscular endurance tests, at any load, such

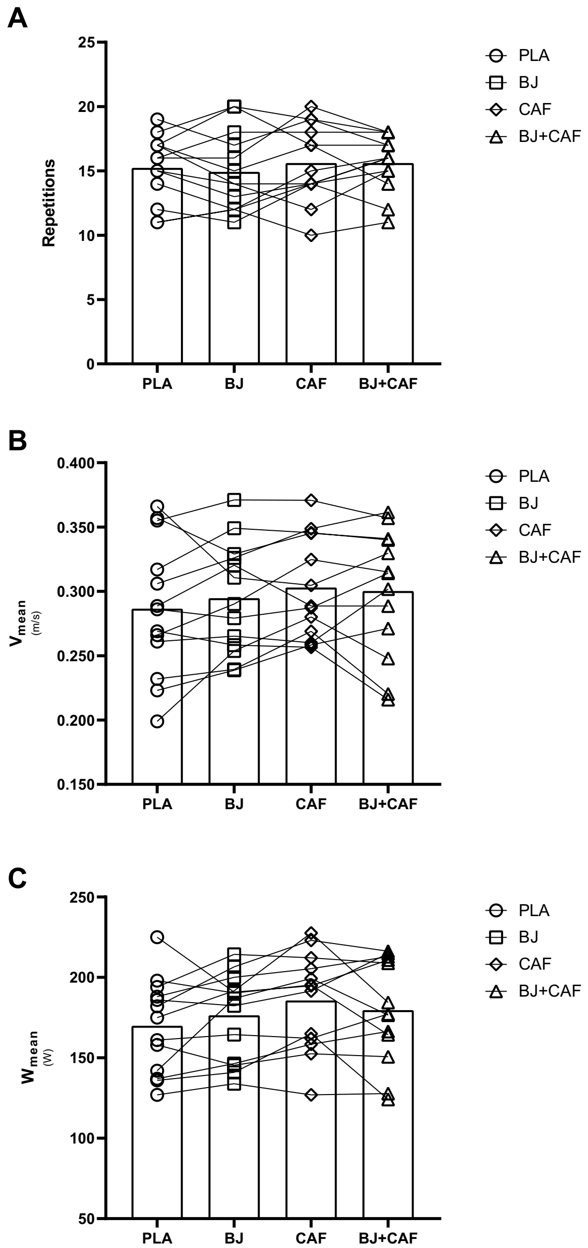

Fig. 3. Muscular endurance test differences in the number of repetitions, mean velocity and power output among experimental conditions in the bench press exercise. Number of repetitions (**A**), mean velocity (V_{mean}) (**B**) and mean power output (W_{mean}) performed in the bench press exercise (**C**). *PLA* placebo, *BJ* beetroot juice, *CAF* caffeine, *BJ + CAF* beetroot juice and caffeine.

as velocity and power production or time under tension. Only a couple of studies explored this idea, reporting an increase in the number of repetitions, velocity and power production at 65 and 85% 1RM in bench press[24] and 65% and 85% 1RM in back squat exercise[19,27]. Our results are aligned with this evidence, observing an increase in the number of repetitions of V_{mean} and W_{mean} in the back squat exercise at 65%1RM. Prior studies suggest that caffeine's ergogenic effects may be more pronounced in larger muscle groups, such as the quadriceps during back squat compared to the pectoralis major during bench press exercises[19,27], likely due to enhanced central nervous system stimulation that increases motor unit recruitment[40]. However, it cannot be ruled out that caffeine's mechanisms of action may also involve peripheral factors, such as the inhibition of phosphodiesterase, stimulation of calcium ion (Ca^{2+}) release from the sarcoplasmic reticulum, increased sodium–potassium pump activity, and antagonism of benzodiazepine receptors in skeletal muscle[41–43]. Future studies are required to further clarify the relative contributions of central and peripheral mechanisms to caffeine's ergogenic effects.

Interestingly, in the muscular endurance test, beetroot juice supplementation also stimulates an ergogenic effect by increasing the number of repetitions, V_{mean} and W_{peak}, even though this effect was found in the back squat but not in the bench press exercise. Previous studies have reported conflicting results on this topic, some

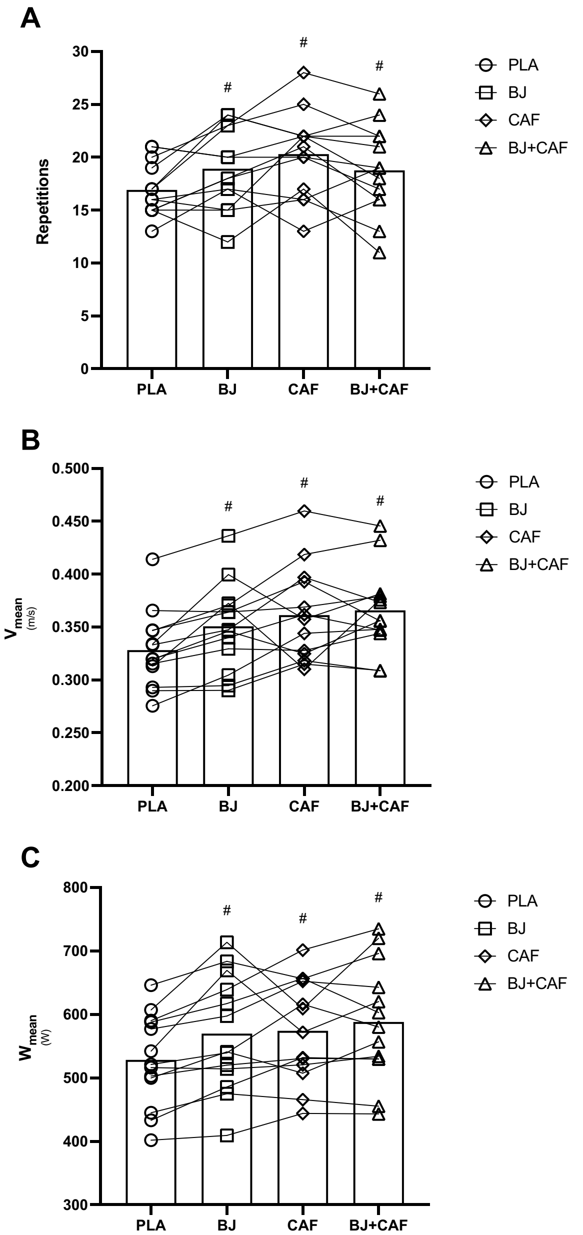

Fig. 4. Muscular endurance test differences in the number of repetitions, mean velocity and power output among experimental conditions in the back squat exercise. Number of repetitions (**A**), mean velocity (V_{mean}) (**B**) and mean power output (W_{mean}) performed in the back squat exercise (**C**). $^{\#}P < 0.05$ compared to PLA. *PLA* placebo, *BJ* beetroot juice, *CAF* caffeine, *BJ + CAF* beetroot juice and caffeine.

of them reporting that low (~6 mmol/L of NO_3^-) to moderate (~12 mmol/L of NO_3^-) doses of BJ stimulate an increase in one set of bench press or back squat exercise performing until task failure[29,31,44,45] but not in others[29,46,47]. In a meta-analysis, Evangelista et al.[48] indicated that the acute intake of 316–985 mg of BJ during the 2–3 h before the trial can cause a significant effect in strength production when this is measured under fatigue conditions but not in a resting state. In fact, Tan et al.[47] found that 11.8 mmol NO_3^- increases muscular endurance in one set of repetitions-to-failure in bench press when this exercise was preceded by the same test performed in back squat exercise. In our study, the order of the muscular endurance tests was inverted and the back squat was preceded by the bench press exercise, reporting an increase in performance in the second muscular endurance test as the one reported by Tan et al.[47] despite using a lower dose of NO_3^- (6.5 mmol/L). Prior fatigue of a remote muscle group can expedite fatigue in another muscle group an effect linked to a greater central fatigue development[49]. Therefore, despite that dietary NO_3^- supplementation does not appear to alter central fatigue development during cycling exercise[50,51], in resistance exercises NO_3^- supplementation may mitigate central fatigue. In addition, this idea is also consistent with the notion that NO_3^- supplementation preferentially enhances type II muscle fiber recruitment[6]. Although upper-body muscles may contain a higher proportion of type II fibers compared to lower-body muscles[52], during tasks performed to failure, type II fibers are recruited regardless of load or repetition duration[53]. Nonetheless, further research is needed to determine the mechanism by which NO_3^- can cause an acute ergogenic effect after accumulated neuromuscular fatigue in muscular endurance.

The main novel finding of the present study is that not only the single but the combined caffeine and beetroot juice supplementation increase muscular endurance performance. In back squat exercise, although no differences were found in the number of repetitions performed, which were similar among experimental conditions: caffeine and beetroot juice (19 ± 2 repetitions), beetroot juice (18 ± 3 repetitions) and caffeine (18 ± 3 repetitions); we observed that compared to the placebo condition, the V_{mean} and W_{mean} increase after caffeine and beetroot juice intake (11.5% and 11.3%) was slightly greater than the single intake of beetroot juice (6.8% and 7.8%, respectively) and caffeine (10% and 8.6%, respectively). Although the combination of BJ and CAF led to small, non-significant improvements in V_{mean} and W_{mean} compared to either supplement alone (1.5–2.7%), these differences may reflect true but undetected effects due to limited statistical power and individual variability. Repetition count to failure, however, did not differ across conditions, suggesting that enhancements in power output may not necessarily translate into improved muscular endurance. This discrepancy may be explained by the distinct physiological determinants of each measure (e.g., peripheral fatigue tolerance, substrate availability or neuromuscular efficiency) and may reflect that V_{mean} and W_{mean} may be more sensitive indicators of subtle ergogenic effects than repetition count alone. Therefore, to our knowledge, no previous study has evaluated the potential additive effect of these supplements, particularly in resistance exercise. Consequently, although future studies with larger sample sizes and more fatigue-sensitive protocols may help clarify the potential additive effects of combined supplementation, the co-ingestion of caffeine and beetroot juice may constitute an effective dietary supplement protocol under conditions where fatigue affects the skeletal muscles' ability to preserve force and power production during repeated muscle contraction.

Limitations

Finally, while this study provides novel insights into the combined and isolated effects of beetroot juice and caffeine on resistance exercise performance, some limitations should be acknowledged. A major limitation was the inability to measure plasma levels of nitrate and caffeine. This restricts our ability to confirm whether the supplementation protocols effectively elevated systemic concentrations of these substances, or to verify individual variability in absorption and metabolism. Consequently, we cannot entirely rule out differences in pharmacokinetics that might have influenced the ergogenic responses observed.

Additionally, although participants were instructed to replicate their diet and abstain from foods rich in nitrates and caffeine, as well as to avoid oral hygiene practices that might interfere with nitrate reduction, we cannot completely eliminate the possibility of residual dietary intake or differences in oral microbiota that could act as confounding factors. Nonetheless, rigorous control measures, such as a triple-blind, randomized, and crossover design, strict pre-trial dietary restrictions, and standardized testing protocols, were implemented to minimize potential biases and variability.

Finally, it should be noted that the fixed testing sequence, with muscular endurance preceding strength/power assessments, may have introduced a degree of fatigue that could potentially influence subsequent performance outcomes, despite standardized rest periods.

Future studies including blood sampling to confirm plasma nitrate and caffeine concentrations would strengthen the interpretation of the physiological responses to these supplements. Additionally, it would be beneficial to explore the combined effects of these supplements in larger sample sizes, exploring different populations (e.g., female athletes or non-resistance-trained individuals) and varying dosages of these supplements (e.g., 16.8 mmol of nitrates and 6 mg/kg of caffeine).

Conclusions

In conclusion, acute caffeine but not beetroot juice intake improves velocity and power production, particularly at high loads (75–100%1RM) and in lower-body (i.e., back squat) but not in upper-body exercises (i.e., bench press). In contrast, the single but particularly combined acute intake of beetroot juice and caffeine increase the number of repetitions, mean velocity and power production in muscular endurance at 65%1RM in back squat exercise. Therefore, in resistance-trained males, the combined beetroot juice and caffeine intake may produce a summative effect in fatiguing tasks where velocity and power production are required to be maintained.

Practical applications

The findings of this study have important implications for sports nutrition and resistance training practices. Firstly, acute caffeine intake at a moderate dose (3 mg/kg) can enhance muscular strength and power, particularly in lower-body exercises at high loads (75–100% 1RM). Thus, athletes aiming to maximize peak force production and velocity during strength-focused training or competition might benefit from strategic caffeine supplementation.

Secondly, both beetroot juice (6.5 mmol NO_3^-) and caffeine supplementation improved muscular endurance performance during back squat exercises at 65% 1RM, with combined supplementation producing a slightly superior effect on maintaining mean velocity and power output under fatigue. Therefore, in disciplines requiring repeated high-intensity efforts (e.g., CrossFit or rugby), combined ingestion of beetroot juice and caffeine before competition or training could help maintain performance under conditions of muscular fatigue.

Given that no improvements were observed in upper-body exercises (bench press), these strategies should be specifically recommended for activities with a greater reliance on lower-body strength and endurance. Finally, athletes and coaches should consider timing the supplementation appropriately (e.g., caffeine 60 min and beetroot juice 180 min before activity) to optimize ergogenic effects.

Methods

Participants

Thirteen male resistance-trained individuals (age, 23.8 ± 4.9 years; body mass, 73.4 ± 7.8 kg; training experience, 4.6 ± 3.3 years and 4.6 ± 1.3 sessions/week; bench press (1RM/kg body mass), 1.25 ± 0.3; back squat (1RM/kg body mass), 1.85 ± 0.4) participated in this study being recruited in a 2 month period of time.

The inclusion/exclusion criteria for this study were as follows: (a) participants aged between 18 and 35 years; (b) absence of neuromuscular, musculoskeletal, neurological, immunological, or cardio-metabolic disorders; (c) a minimum of 8 months of experience in resistance training, with a training frequency of at least 3 days per week over the past 3 months, as confirmed by a questionnaire; (d) no use of tobacco[54], vaper, or taking any medication known to interfere with stomach acid production, drug, stimulant or any other sports supplement during the trial that may interfere neuromuscular performance. Consumption of caffeine was not considered an inclusion or exclusion criterion; however, in the pre-trial 24-h recall, participants reported a caffeine intake of 6.9 ± 6.2 mg/kg/day, classifying them as high caffeine consumers[55].

Before study enrolment, all procedures potential risks or discomfort associated with the experiments were explained to participants, and after solving any doubts related to the experiment, they gave their written informed consent. The study design and protocol adhered to the tenets of the Declaration of Helsinki and was approved by the University Ethical Committee of Investigation (CEIP/2023/4/093) and registered at ClinicalTrial.gov (NCT06596395).

Experimental design

The study design was randomized, triple-blind, cross-over and placebo-controlled. Participants reported to the laboratory on 5 occasions at the same time of day (± 0.5 h) to avoid the potential influence of circadian rhythms on neuromuscular performance. During the first visit, participants underwent preliminary questionnaires of dietary and physical activity habits and body composition assessments, and a familiarization session where they experienced all tests performed in the trials. During visits two to five, participants were assigned to four conditions: (a) Caffeine (CAF); (b) Beetroot juice (BJ); (c) Caffeine plus beetroot juice (CAF + BJ); (d) Placebo (PLA). The experimental protocol is illustrated in Supplementary Fig. 1. Each trial was separated by 3 to 7 days. The order of the trials was randomized according to each participant's experimental condition (www.randomized.org). An external researcher elaborated the alphanumeric code assigned to each sequence to blind participants and researchers during the trials. The codes were unveiled after statistical analysis to blind statistician.

Experimental protocol

Body composition, dietary and physical activity habits

Body composition was assessed using electric bioimpedance (Tanita MC-780MA, Tanita Corporation of America Inc. IL, USA). Dietary habits were analyzed using a 24-h dietary recall (i.e., Spanish Food Composition Database (BEDCA) and CESNID Food composition data tables) while physical activity habits were evaluated using the International Physical Activity Questionnaire (IPAQ). Dietary habits (energy intake, 2489 ± 759 kcal; protein, 1.92 g/kg; carbohydrate, 3.33 g/kg; fat, 1.28 g/kg), physical activity habits (physical activity, 4983 ± 457 METs-min/week; sedentary time, 6.4 ± 2.8 h/day) were replicated in the 24 h before each trial. Moreover, 24 h before the familiarization session and until the end of the trial, participants were encouraged to refrain from stimulants and alcohol intake. Participants were also instructed to abstain from all caffeine-containing foods, beverages, and supplements for 24 h before each testing session to minimize potential variability related to habitual caffeine intake and to standardize baseline caffeine levels across conditions. Besides, Dietary NO_3^- intake was restricted by providing subjects with a list of foods rich in NO_3^- (e.g., beetroot, celery, or spinach) that they should avoid in the 48 h before each trial session. Participants were encouraged to avoid brushing their teeth or using any oral antiseptic rinse, or chewing gum or ingesting sweets that could alter their oral microbiota and interfere with NO_3^- reduction during the 24 h leading up to each experimental trial[56].

Supplementation protocol

The supplementation protocol started 180 min before the trial. Participants ingested 70 mL of concentrated NO_3^--rich (BJ, 6.5 mmol NO_3^-) or NO_3^--depleted (PLA, ~ 0.04 mmol NO_3^-) beetroot juice (Beet IT; James White Drinks Ltd., Ipswich, UK). Then, 60 min before the trial, participants ingested caffeine (CAF, 3 mg/kg, HSN, Granada, Spain) or placebo (PLA, 3 mg/kg, maltodextrin), dissolved in 150 ml of tap water adding a flavoring with no calories to mask the supplements' flavor and smell (MyProtein, Northwich, UK). The beverages were provided in opaque shaker bottles.

One-repetition maximum (1RM)

1-repetition maximum (1RM) of bench press and back squat exercises were obtained during the first visit to determine the load (kg) corresponding to 25%, 50%, 75%, 90% and 100% 1RM for each participant in a Smith machine (Multipower, Technogym, Spain). The 1RM protocol was initiated with a load set at 20 kg. This load was increased by 15–10 kg until mean velocity (V_{mean}) reached 0.2 m/s in bench press and 0.4 m/s in back squat using a linear transductor (Encoder, Chronojump Boscosystem, Spain)[19]. Then, smaller increments (< 5 kg) were adjusted to determine the 1RM. After 20 min of passive recovery, participants carried out a familiarization session, performing the same test in the same order as in the experimental trials.

Muscular strength and power

After a standardized warm-up of 10 min of dynamic stretches and joint mobilization exercises, the participants initiated the muscular strength and power test. The test consisted of the measurement of bar velocity displacement in a Smith machine (Multipower, Technogym, Spain), with a linear encoder attached to the bar

(Encoder, Chronojump Boscosystem, Spain) to measure muscular mean, peak and time to reach peak velocity (V_{mean}, V_{peak} and Time to V_{peak}) and power output (W_{mean}, W_{peak} and Time to W_{peak}) at five incremental loads 25%, 50%, 75%, 90% and 100% 1RM for bench press and back squat exercises. On each trial, three attempts were executed to 25%1RM, two to 50%1RM and one for 75%, 90% and 100% 1RM. On each attempt, participants were instructed to perform the eccentric phase in a controlled manner, pause for 2 s in the isometric phase, and then execute the concentric phase at the maximal velocity possible, ensuring a similar range of movement for each exercise[57]. Three minutes of passive recovery were allowed between sets and exercises.

Muscular endurance
Participants were required to complete one set at 65% 1RM in bench press and back squat exercise, performing as many repetitions as possible until task failure. The order of the exercise types and loads was always the same on each trial. On each attempt, participants were instructed to perform the eccentric phase in a controlled manner, pause for 2 s in the isometric phase, and then execute the concentric phase at maximum velocity, ensuring a similar range of movement for each exercise. Each set or exercise was interposed by 5 min of passive recovery. The number of repetitions, as well as mean, peak and time to reach peak velocity and power output were obtained from each repetition and averaged. On each participant, the number of repetitions selected to average velocity and power variables were the lower performed on any of the experimental conditions.

Isometric strength and vertical jump test
The isometric handgrip and isometric mid-thigh pull tests were performed using handgrip and back/legs dynamometers (Grip-D, Takei, Japan). Each test was repeated two times, maintaining maximal muscular tension for five seconds on each attempt and allowing 30 s of passive recovery and the best attempt performed was recorded for subsequent analysis.

Vertical jump ability was assessed using countermovement jump (CMJ, without arm swing) tests performed on a force platform (Kistler 9229A, Winterthur, Switzerland). Participants completed three attempts for each test, allowing one minute of passive recovery and recording the average and best attempt performed by each participant.

Questionnaires and scales
At the end of the familiarization and the trials, participants were required to fill out a questionnaire about their perception of power, endurance, energy and exertion, as well as heart, muscular and gastrointestinal discomfort. This questionnaire included a 1- to 5-point scale to assess each item. Participants were previously informed that 1 point meant the minimal amount of that item and 5 points meant the maximal amount of the item. Moreover, Participants' mood was assessed using a reduced version of the profile of mood states questionnaire (POMS)[20]. Participants graded a set of 29 items related to the mood on a Likert scale from 0 (not at all) to 4 (extremely) in reply to the question "How do you feel at this moment?" to assess six scales: tension, depression, anger, vigor, fatigue and confusion. Additionally, a specific question to evaluate the blinding procedure was also included.

Statistical analysis
The sample size calculation revealed that 12 participants were sufficient for the purpose of the study to show an effect size of 0.45 ($\alpha = 0.05$; $1 - \beta = 0.80$) (v3.1, G*power, Dusseldorf University, Germany); finally, 14 participants were recruited and finally 13 took part in the investigation.

Data collected in the study were analyzed using the statistical package SPSS v29.0 (SPSS Inc., Chicago, IL, USA) and figures were generated using GraphPad Prism (v8, GraphPad Software Inc., La Jolla, CA, USA). Firstly, the Shapiro–Wilk test was used to assess whether the data followed a normal distribution. At a significance level (α) of 0.05, the resulting p-value was greater than 0.05, indicating that the assumption of normality was not violated. Muscular strength/power was analyzed using a two-way ANOVA for repeated measures according to supplements (CAF, BJ, CAF + BJ and PLA), load (25, 50, 75, 90 and 100%1RM) for each exercise type. Muscular endurance was analyzed using a one-way ANOVA for repeated measures according to supplement (CAF, BJ, CAF + BJ and PLA) using the only load measured (65%1RM) for each exercise type. Mauchly's test of sphericity was conducted to assess the assumption of sphericity required for repeated-measures ANOVA. When the assumption of sphericity was violated (i.e., $P < 0.05$), the degrees of freedom were corrected using the Greenhouse–Geisser adjustment. In cases where the Greenhouse–Geisser epsilon exceeded 0.75, the Huynh–Feldt correction was applied. Holm-Bonferroni correction was used as a post hoc test when the main or interaction effect was identified in the ANOVA and when pairwise comparisons were conducted. Finally, the McNemar test was also used to detect differences in side effects after beverage intake.

Values are reported as mean ± standard deviation (SD). The significance level was set at $P < 0.05$. Effect size (ES) was calculated as partial eta squared statistic (η_p^2) for the two-way repeated measures and Hedges's (g) for partial comparisons.

Data availability
The dataset used and analyzed during the current study is available from the corresponding author on reasonable request.

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References

1. Guest, N. S. et al. International society of sports nutrition position stand: Caffeine and exercise performance. *J. Int. Soc. Sports Nutr.* **18**, 1. https://doi.org/10.1186/s12970-020-00383-4 (2021).
2. Aguilar-Navarro, M. et al. Urine caffeine concentration in doping control samples from 2004 to 2015. *Nutrients*. https://doi.org/10.3390/nu11020286 (2019).
3. Anderson, O. K., Martinez-Ferran, M., Lorenzo-Calvo, J., Jimenez, S. L. & Pareja-Galeano, H. Effects of nitrate supplementation on muscle strength and mass: A systematic review. *J. Strength Cond. Res.* **36**, 3562–3570. https://doi.org/10.1519/JSC.0000000000004101 (2022).
4. Sanchez-Oliver, A. J., Grimaldi-Puyana, M. & Dominguez, R. Evaluation and behavior of Spanish bodybuilders: Doping and sports supplements. *Biomolecules* https://doi.org/10.3390/biom9040122 (2019).
5. Bailey, S. J. et al. Inorganic nitrate supplementation improves muscle oxygenation, O(2) uptake kinetics, and exercise tolerance at high but not low pedal rates. *J. Appl. Physiol.* **1985**(118), 1396–1405. https://doi.org/10.1152/japplphysiol.01141.2014 (2015).
6. Jones, A. M. Influence of dietary nitrate on the physiological determinants of exercise performance: A critical review. *Appl. Physiol. Nutr. Metab.* **39**, 1019–1028. https://doi.org/10.1139/apnm-2014-0036 (2014).
7. Fulford, J. et al. Influence of dietary nitrate supplementation on human skeletal muscle metabolism and force production during maximum voluntary contractions. *Pflugers Arch.* **465**, 517–528. https://doi.org/10.1007/s00424-013-1220-5 (2013).
8. Bailey, S. J. et al. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J. Appl. Physiol.* **1985**(109), 135–148. https://doi.org/10.1152/japplphysiol.00046.2010 (2010).
9. Jones, A. M., Ferguson, S. K., Bailey, S. J., Vanhatalo, A. & Poole, D. C. Fiber Type-specific effects of dietary nitrate. *Exerc. Sport Sci. Rev.* **44**, 53–60. https://doi.org/10.1249/JES.0000000000000074 (2016).
10. Esen, O., Faisal, A., Zambolin, F., Bailey, S. J. & Callaghan, M. J. Effect of nitrate supplementation on skeletal muscle motor unit activity during isometric blood flow restriction exercise. *Eur. J. Appl. Physiol.* **122**, 1683–1693 (2022).
11. Wylie, L. J. et al. Dietary nitrate supplementation improves team sport-specific intense intermittent exercise performance. *Eur. J. Appl. Physiol.* **113**, 1673–1684 (2013).
12. Lopes, J. M., Aubier, M., Jardim, J., Aranda, J. V. & Macklem, P. T. Effect of caffeine on skeletal muscle function before and after fatigue. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **54**, 1303–1305. https://doi.org/10.1152/jappl.1983.54.5.1303 (1983).
13. Allen, D. G., Lamb, G. D. & Westerblad, H. Impaired calcium release during fatigue. *J. Appl. Physiol.* **1985**(104), 296–305. https://doi.org/10.1152/japplphysiol.00908.2007 (2008).
14. Glaister, M., Pattison, J. R., Muniz-Pumares, D., Patterson, S. D. & Foley, P. Effects of dietary nitrate, caffeine, and their combination on 20-km cycling time trial performance. *J. Strength Cond. Res.* **29**, 165–174. https://doi.org/10.1519/JSC.0000000000000596 (2015).
15. Handzlik, M. K. & Gleeson, M. Likely additive ergogenic effects of combined preexercise dietary nitrate and caffeine ingestion in trained cyclists. *ISRN Nutr.* **2013**, 396581. https://doi.org/10.5402/2013/396581 (2013).
16. Lane, S. C. et al. Single and combined effects of beetroot juice and caffeine supplementation on cycling time trial performance. *Appl. Physiol. Nutr. Metab.* **39**, 1050–1057. https://doi.org/10.1139/apnm-2013-0336 (2014).
17. Oskarsson, J. & McGawley, K. No individual or combined effects of caffeine and beetroot-juice supplementation during submaximal or maximal running. *Appl. Physiol. Nutr. Metab.* **43**, 697–703. https://doi.org/10.1139/apnm-2017-0547 (2018).
18. Cuenca, E. et al. Effects of beetroot juice supplementation on performance and fatigue in a 30-s all-out sprint exercise: A randomized double-blind cross-over study. *Nutrients* https://doi.org/10.3390/nu10091222 (2018).
19. Ruiz-Fernandez, I., Valades, D., Dominguez, R., Ferragut, C. & Perez-Lopez, A. Load and muscle group size influence the ergogenic effect of acute caffeine intake in muscular strength, power and endurance. *Eur. J. Nutr.* **62**, 1783–1794. https://doi.org/10.1007/s00394-023-03109-9 (2023).
20. Jodra, P. et al. Effects of caffeine supplementation on physical performance and mood dimensions in elite and trained-recreational athletes. *J. Int. Soc. Sports Nutr.* **17**, 2. https://doi.org/10.1186/s12970-019-0332-5 (2020).
21. Grgic, J. Effects of caffeine on resistance exercise: A review of recent research. *Sports Med.* **51**, 2281–2298. https://doi.org/10.1007/s40279-021-01521-x (2021).
22. Grgic, J. & Mikulic, P. Effects of caffeine on rate of force development: A meta-analysis. *Scand. J. Med. Sci. Sports.* https://doi.org/10.1111/sms.14109 (2021).
23. Raya-Gonzalez, J. et al. Acute effects of caffeine supplementation on movement velocity in resistance exercise: A systematic review and meta-analysis. *Sports Med.* **50**, 717–729. https://doi.org/10.1007/s40279-019-01211-9 (2020).
24. Grgic, J. et al. CYP1A2 genotype and acute effects of caffeine on resistance exercise, jumping, and sprinting performance. *J. Int. Soc. Sports Nutr.* **17**, 21. https://doi.org/10.1186/s12970-020-00349-6 (2020).
25. Pallares, J. G. et al. Neuromuscular responses to incremental caffeine doses: Performance and side effects. *Med. Sci. Sports Exerc.* **45**, 2184–2192. https://doi.org/10.1249/MSS.0b013e31829a6672 (2013).
26. Mora-Rodriguez, R. et al. Improvements on neuromuscular performance with caffeine ingestion depend on the time-of-day. *J. Sci. Med. Sport* **18**, 338–342. https://doi.org/10.1016/j.jsams.2014.04.010 (2015).
27. Montalvo-Alonso, J. J. et al. Sex differences in the ergogenic response of acute caffeine intake on muscular strength, power and endurance performance in resistance-trained individuals: A randomized controlled trial. *Nutrients* https://doi.org/10.3390/nu16111760 (2024).
28. Haider, G. & Folland, J. P. Nitrate supplementation enhances the contractile properties of human skeletal muscle. *Med. Sci. Sports Exerc.* **46**, 2234–2243. https://doi.org/10.1249/MSS.0000000000000351 (2014).
29. Ranchal-Sanchez, A. et al. Acute effects of beetroot juice supplements on resistance training: A randomized double-blind crossover. *Nutrients* https://doi.org/10.3390/nu12071912 (2020).
30. Williams, T. D., Martin, M. P., Mintz, J. A., Rogers, R. R. & Ballmann, C. G. Effect of acute beetroot juice supplementation on bench press power, velocity, and repetition volume. *J. Strength Cond. Res.* **34**, 924–928. https://doi.org/10.1519/JSC.0000000000003509 (2020).
31. Jurado-Castro, J. M., Campos-Perez, J., Ranchal-Sanchez, A., Duran-Lopez, N. & Dominguez, R. Acute effects of beetroot juice supplements on lower-body strength in female athletes: Double-blind crossover randomized trial. *Sports Health* **14**, 812–821. https://doi.org/10.1177/19417381221083590 (2022).
32. Rodriguez-Fernandez, A., Castillo, D., Raya-Gonzalez, J., Dominguez, R. & Bailey, S. J. Beetroot juice supplementation increases concentric and eccentric muscle power output. Original investigation. *J. Sci. Med. Sport* **24**, 80–84. https://doi.org/10.1016/j.jsams.2020.05.018 (2021).
33. Coggan, A. R. et al. Dietary nitrate-induced increases in human muscle power: high versus low responders. *Physiol. Rep.* https://doi.org/10.14814/phy2.13575 (2018).
34. Warren, G. L., Park, N. D., Maresca, R. D., McKibans, K. I. & Millard-Stafford, M. L. Effect of caffeine ingestion on muscular strength and endurance: A meta-analysis. *Med. Sci. Sports Exerc.* **42**, 1375–1387. https://doi.org/10.1249/MSS.0b013e3181cabbd8 (2010).
35. Ferreira, T. T., da Silva, J. V. F. & Bueno, N. B. Effects of caffeine supplementation on muscle endurance, maximum strength, and perceived exertion in adults submitted to strength training: A systematic review and meta-analyses. *Crit. Rev. Food Sci. Nutr.* **61**, 2587–2600. https://doi.org/10.1080/10408398.2020.1781051 (2021).
36. Grgic, J. et al. What dose of caffeine to use: Acute effects of 3 doses of caffeine on muscle endurance and strength. *Int. J. Sports Physiol. Perform.* https://doi.org/10.1123/ijspp.2019-0433 (2019).

37. Norum, M. et al. Caffeine increases strength and power performance in resistance-trained females during early follicular phase. *Scand. J. Med. Sci. Sports* **30**, 2116–2129. https://doi.org/10.1111/sms.13776 (2020).
38. Salatto, R. W., Arevalo, J. A., Brown, L. E., Wiersma, L. D. & Coburn, J. W. Caffeine's effects on an upper-body resistance exercise workout. *J. Strength Cond. Res.* **34**, 1643–1648. https://doi.org/10.1519/JSC.0000000000002697 (2020).
39. Polito, M. D., Grandolfi, K. & de Souza, D. B. Caffeine and resistance exercise: the effects of two caffeine doses and the influence of individual perception of caffeine. *Eur. J. Sport Sci.* **19**, 1342–1348. https://doi.org/10.1080/17461391.2019.1596166 (2019).
40. Bazzucchi, I., Felici, F., Montini, M., Figura, F. & Sacchetti, M. Caffeine improves neuromuscular function during maximal dynamic exercise. *Muscle Nerve* **43**, 839–844. https://doi.org/10.1002/mus.21995 (2011).
41. Davis, J. K. & Green, J. M. Caffeine and anaerobic performance: Ergogenic value and mechanisms of action. *Sports Med.* **39**, 813–832. https://doi.org/10.2165/11317770-000000000-00000 (2009).
42. Penner, R., Neher, E., Takeshima, H., Nishimura, S. & Numa, S. Functional expression of the calcium release channel from skeletal muscle ryanodine receptor cDNA. *FEBS Lett.* **259**, 217–221. https://doi.org/10.1016/0014-5793(89)81532-7 (1989).
43. James, R. S., Kohlsdorf, T., Cox, V. M. & Navas, C. A. 70 microM caffeine treatment enhances in vitro force and power output during cyclic activities in mouse extensor digitorum longus muscle. *Eur. J. Appl. Physiol.* **95**, 74–82. https://doi.org/10.1007/s00421-005-1396-2 (2005).
44. Garnacho-Castano, M. V. et al. Circulating nitrate-nitrite reduces oxygen uptake for improving resistance exercise performance after rest time in well-trained CrossFit athletes. *Sci. Rep.* **12**, 9671. https://doi.org/10.1038/s41598-022-13786-x (2022).
45. Mosher, S. L., Sparks, S. A., Williams, E. L., Bentley, D. J. & McNaughton, L. R. Ingestion of a nitric oxide enhancing supplement improves resistance exercise performance. *J. Strength Cond. Res.* **30**, 3520–3524. https://doi.org/10.1519/JSC.0000000000001437 (2016).
46. Flanagan, S. D. et al. The effects of nitrate-rich supplementation on neuromuscular efficiency during heavy resistance exercise. *J. Am. Coll. Nutr.* **35**, 100–107. https://doi.org/10.1080/07315724.2015.1081572 (2016).
47. Tan, R. et al. Effects of dietary nitrate supplementation on performance and muscle oxygenation during resistance exercise in men. *Nutrients* https://doi.org/10.3390/nu14183703 (2022).
48. Evangelista, J. F., Meirelles, C. M., Aguiar, G. S., Alves, R. & Matsuura, C. Effects of beetroot-based supplements on muscular endurance and strength in healthy male individuals: A systematic review and meta-analysis. *J. Am. Nutr. Assoc.* **43**, 77–91. https://doi.org/10.1080/27697061.2023.2211318 (2024).
49. Johnson, M. A., Sharpe, G. R., Williams, N. C. & Hannah, R. Locomotor muscle fatigue is not critically regulated after prior upper body exercise. *J. Appl. Physiol.* **1985**(119), 840–850. https://doi.org/10.1152/japplphysiol.00072.2015 (2015).
50. Husmann, F., Bruhn, S., Mittlmeier, T., Zschorlich, V. & Behrens, M. Dietary nitrate supplementation improves exercise tolerance by reducing muscle fatigue and perceptual responses. *Front. Physiol.* **10**, 404. https://doi.org/10.3389/fphys.2019.00404 (2019).
51. Thurston, T. S. et al. On the implication of dietary nitrate supplementation for the hemodynamic and fatigue response to cycling exercise. *J. Appl. Physiol.* **1985**(131), 1691–1700. https://doi.org/10.1152/japplphysiol.00400.2021 (2021).
52. Zinner, C. et al. The physiological mechanisms of performance enhancement with sprint interval training differ between the upper and lower extremities in humans. *Front. Physiol.* **7**, 426. https://doi.org/10.3389/fphys.2016.00426 (2016).
53. Morton, R. W. et al. Muscle fibre activation is unaffected by load and repetition duration when resistance exercise is performed to task failure. *J. Physiol.* **597**, 4601–4613. https://doi.org/10.1113/JP278056 (2019).
54. Bailey, S. J. et al. Improvement in blood pressure after short-term inorganic nitrate supplementation is attenuated in cigarette smokers compared to non-smoking controls. *Nitric Oxide* **61**, 29–37. https://doi.org/10.1016/j.niox.2016.10.002 (2016).
55. Filip, A., Wilk, M., Krzysztofik, M. & Del Coso, J. Inconsistency in the ergogenic effect of caffeine in athletes who regularly consume caffeine: Is it due to the disparity in the criteria that defines habitual caffeine intake?. *Nutrients* https://doi.org/10.3390/nu12041087 (2020).
56. Bescos, R. et al. Effects of chlorhexidine mouthwash on the oral microbiome. *Sci. Rep.* **10**, 5254. https://doi.org/10.1038/s41598-020-61912-4 (2020).
57. Pallarés, J. G., Sánchez-Medina, L., Pérez, C. E., De La Cruz-Sánchez, E. & Mora-Rodriguez, R. Imposing a pause between the eccentric and concentric phases increases the reliability of isoinertial strength assessments. *J. Sport Sci.* **32**, 1165–1175. https://doi.org/10.1080/02640414.2014.889844 (2014).

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Author contributions
JJMA and APL conceived the experiment. JJMA, ALS and APL designed the experiment. JJMA, MVM, CF, DV, ALS and APL collected the data. JJMA and APL analyzed and interpreted the data. JJMA, RD and APL drafted the manuscript. All authors read and approved the final version of the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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