Scientific-Expert Journal of Anthropological Aspects of Sports, Physical Education and Recreation

Submitted: 14/07/2024
Accepted: 26/08/2024
Published: 03/09/2024
UDK: 615.357.015.3:577.175.5

Original article

Effect of Acute Beetroot Supplementation on Exercise Performance of CrossFit® Male Athletes: Randomized, Double-blind, Placebo-Controlled Crossover Study

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Abstract

The study aimed to evaluate the effect of beetroot supplementation on the number of repetitions in the Cindy test of male athletes who practice CrossFit® to improve their exercise performance. Twenty male CrossFit® athletes (age = 28 ±3 years, height = 1.71 ± 0.2 m, weight = 72.2 ± 4.2 kg) were included in a randomized, double-blind, placebo-controlled and crossover study. The participants consumed a beetroot supplement (515 mg (8.24 mmol) of nitrate) or placebo (starch) 90 min before starting the Cindy workout. Student's t-tests for paired samples were used to compare the performance between the beetroot supplement and placebo conditions and to examine for a learning effect between the first and the second sessions. No significant differences were found in performance between the beetroot supplement and the placebo conditions (17.4 ± 2.7 vs. 16.7± 2.3 rounds, t = 1.48, p = 0.078). However, a significant learning effect was determined between the first and the second session (16.6 ± 2.4 vs. 17.5± 2.6 rounds, t = 1.94, p= 0.034). Overall, the intake of 515 mg of beetroot nitrate (8.24 mmol) before a Cindy workout did not increase the number of repetitions performed. More studies are needed to confirm whether nitrate in beetroot can be used as an effective performance improvement strategy in CrossFit® male athletes, considering that a learning effect could be present in the practice of CrossFit® workouts.

Keywords: Beetroot, Dietary nitrate, Exercise performance, CrossFit, High-intensity functional training

Citation: Madera et al. (2024). Effect of Acute Beetroot Supplementation on Exercise Performance of CrossFit® Male Athletes: Randomized, Double-blind, Placebo-Controlled Crossover Study. *SportLogia*. *20*(2), 20-27. https://doi.org/10.7251/SGIA2402020M

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Introduction

CrossFit® has been characterized as a type of high-intensity training, with constant variability of functional movements that leads to improved physical conditioning. The exercises performed are usually high-intensity with little-to-no rest periods between sets, combining strength and endurance exercises such as running, Olympic weightlifting, and body weight movements (Dos Santos Quaresma et al., 2021). The number of people competing in CrossFit® games has grown considerably in recent years (CrossFit LLC, 2024). Similar to athletes in other sports, CrossFit® athletes report using a variety of nutritional supplements to enhance athletic performance (de Souza et al., 2021). Dietary nitrate is a popular nutritional supplement which has been shown to have positive effects on exercise performance after consumption (Hlinsky et al., 2020; Senefeld et al., 2020).

Dietary nitrate, found abundantly in vegetables such as beetroot, spinach, and lettuce, is converted in the body to nitric oxide (NO) via the enterosalivary nitrate-nitrite-NO pathway (Liu et al., 2023; Morou-Bermudez et al., 2022). Nitric oxide is known to play a crucial role in regulating blood flow, muscle contractility, and mitochondrial function, all of which are critical to athletic performance (Gantner et al., 2020; Jones et al., 2018; Pappas et al., 2023). Several high-quality systematic reviews and meta-analyses have explored the performance-enhancing benefits of dietary nitrate, the findings of which support that dietary nitrate supplementation, mostly in the form of concentrated beetroot juice, can improve muscle power (Coggan et al., 2021), single or repeated bouts of high-intensity exercise (Alsharif et al., 2023), and endurance exercise performance (Gao et al., 2021).

However, CrossFit® is a unique sports modality that's performance is determined by strength, endurance, flexibility, and body composition (Hollerbach et al., 2021; Tibana et al., 2021), and is performed through a variety of time domains and varying intensities. To date, very few studies have investigated the effects of dietary nitrate on exercise performance specific to CrossFit® (Garnacho-Castano et al., 2022; Kramer et al., 2016). Kramer et al. (2016) evaluated the effect of a 6-day intake of potassium nitrate (8 $mmol \cdot d^{-1}$), on CrossFit® performance (Kramer et al., 2016). Peak power was improved during a Wingate test, but there were no improved elements of strength or endurance in male CrossFit athletes (Kramer et al., 2016). This is perhaps unsurprising given that the final nitrate dose was consumed ≥24 h before performance testing, which is not in line with expert consensus that recommends the final dose ingested 2–4 h pre-exercise in chronic supplementation regimes (Shannon et al., 2022). On the other hand, Garnacho-Castaño et al. (2020) found that acute beetroot juice supplementation (~ 12.8 mmol of nitrate) 3 hours pre-exercise improved the number of repetitions of wall balls and back squats performed with rest. A limitation of these studies is that they did not adopt an established common benchmark workout typically used in CrossFit® but rather characteristic exercises.

Certainly, acute supplementation is more favorable to athletes as it is more cost-effective and practical however based on current research there is limited evidence to support the use of dietary nitrates as an ergogenic supplement for CrossFit®-specific performance. In this context, the aim of this study was to investigate the effects of acute nitrate supplementation on a common benchmark workout in CrossFit® used to track performance improvements. According to previous investigations demonstrating performance improvement in high-intensity intermittent exercise with beetroot supplementation, it was hypothesized that after the intake of 515 mg (8.24 mmol) of nitrate contained in beetroot supplement, the number of rounds performed during the CrossFit® workout test would increase, compared to the number of rounds completed performing the same CrossFit® workout test, but under placebo condition.

Methods

Design and participants

Twenty male CrossFit® athletes took part in the study. The present study used a randomized, double-blind, crossover design and was approved by the bioethics committee of Cuauhtemoc University to carry out the research, with the code UC001-24. All participants were informed of the protocol and the risks that could present after consumption of the supplement, informed consent was obtained, and a written privacy notice was provided to each person to obtain their approval to participate in the present study. Inclusion criteria were: 1) male between 18 and 40 years of age; 2) at least 6 months of CrossFit® experience; 3) who did not consume supplements of any kind, except protein, or any medication; 4) who were not allergic to beetroot; and 5) without any diagnosed diseases. The sample size was based on previous similar studies (Oliveira et al., 2023; Stein et al., 2020; Ziyaiyan et al., 2023) resulting in a total of 20 participants, who were needed to determine the effects of beetroot supplementation for CrossFit® athletes. For this study, no instructions were given on diet, alcohol, and caffeine consumption, because these values were not standardized.

Measurements and Procedures

The study began with an interview with the volunteers to verify the selection criteria, in addition to explaining the protocol, the Cindy test and the risks that could occur during the protocol. Once informed, all participants signed the privacy notice and the informed consent form in both English and Spanish, thus establishing themselves as study volunteers.

During the screening visit, measurements were taken according to ISAK L1, to estimate the somatotype of the participants. Weight was determined using a digital scale (HBF-514C, Tanita, Japan). Height was measured with a stadiometer, and skinfolds were measured with a picometer (Slime Guide, USA). In the first testing visit, participants were randomized to consume either a beetroot supplement or a placebo. To determine the order of treatment (placebo and then beetroot vs. beetroot and then placebo), participants were randomly assigned using an Excel sheet, with odd numbers being placed into the placebo condition and even numbers being placed into the beetroot condition for the first testing visit (placebo then beetroot: n = 10; beetroot then placebo: n = 10). The project leader was the only investigator who was aware of the randomization of the supplement and placebo; therefore, he was not present during the Cindy test. The volunteers remained blinded throughout the study as well as the researchers responsible for the outcome measurements and the data analysis.

Participants began a self-selected warm-up 60 min after the consumption of the pill(s). Participants began the CrossFit® workout Cindy 90 min after the consumption of the pill(s). The time, 90 min, was selected based on the recommendations of a systematic review evaluating evidence from 23 clinical trials investigating the effect of beetroot supplementation on cardiorespiratory endurance in athletes (Dominguez et al., 2017). The training took place at the facilities of the Universidad Cuauhtémoc Aguascalientes, in an area without a roof, with shade. Each participant performed the workout alone, with no clock or timing device visible to them. CrossFit® training music was played for the participants and was consistent for all of them during their visits. The Cindy workout was chosen because it is standardized by CrossFit® and has been used previously in research (Oliveira et al., 2023; Stein et al., 2020; Ziyaiyan et al., 2023). Participants completed as many rounds as possible of 5 pull-ups, 10 push-ups and 15 squats in 20 minutes. The ranges of motion were established under CrossFit® criteria: kipping was allowed for the pull-ups; push-ups were performed on the toes, with the subject lowering the body straight down until the chest touched the ground and then pushed back up until their elbows locked; squats required subjects to reach full knee and hip extension at the top of each repetition and the hip crease to be below the knee at the bottom of each repetition (Stein et al., 2020). A trainer acted as a judge to measure the movements of the test for each repetition. Repetitions that did not meet movement standards were not counted, and participants were provided with feedback to meet the movement standards. CrossFit® performance was the total number of repetitions completed in 20 min.

Participants were given a post-exercise survey to determine if they perceived an effect from the supplement given (yes/no). A minimum of two weeks were considered for the washout. Participants returned to the University after 2 weeks (wash-out period) (Wylie et al., 2016) of taking the pill(s) and consumed the opposite of the first visit (placebo n = 10; beetroot n = 10) and followed the same test guidelines. After completion of the trial, contact was maintained with participants so that they could report any side effects from taking the pill(s). The experimental design is illustrated in Figure 1 and CONSORT flow chart is shown in Figure 2.

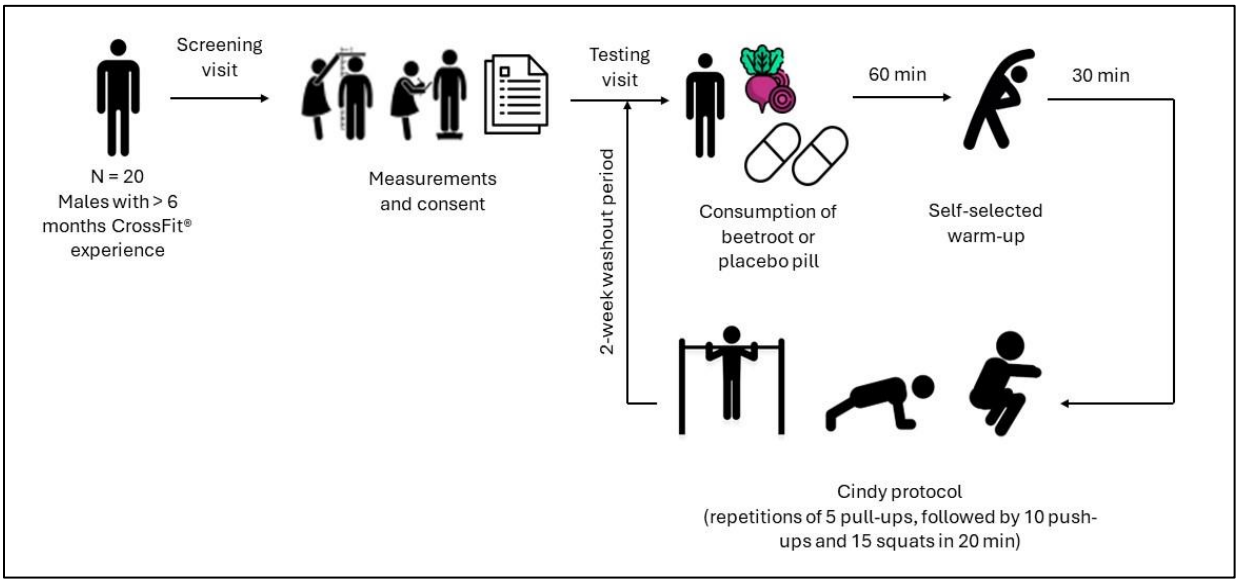

Figure 1. Experimental design.

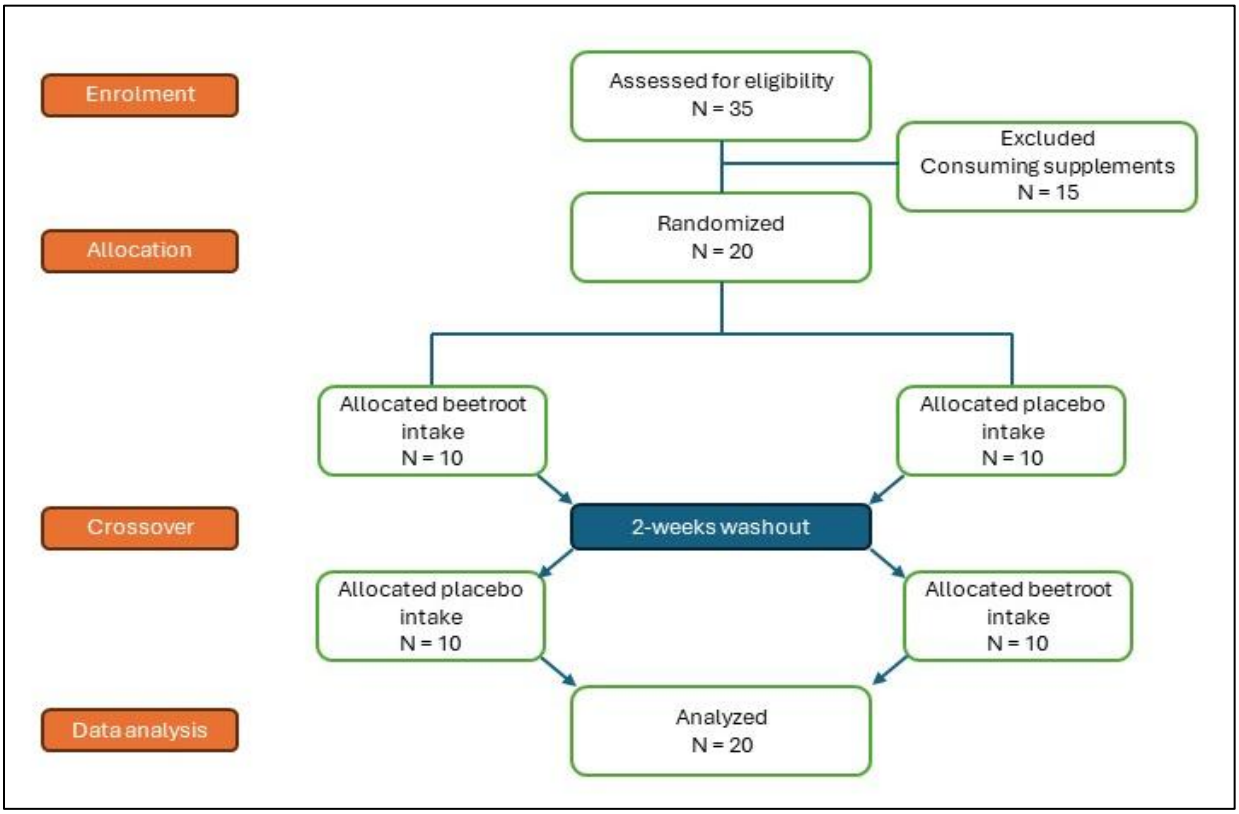

Figure 2. CONSORT flow diagram.

Beetroot supplement and placebo

The supplement used in the protocol was pills of the brand FUTUREBIOTICS with a beetroot content of 515 mg of nitrate (8.24 mmol), which was analyzed using the spectrophotometric technique to determine the amount of nitrates (Brzezinska-Rojek et al., 2023). The placebo was prepared in the Nutrition laboratory of the Universidad Cuauhtémoc Aguascalientes, using 0.4 g of starch and 3 drops of #40 (E-129) and #3 red dye (E-127) in conjunction with two drops of pink vegetable (after the mixture of yellow #3 (E-122) and #6 (E-110), blue #1 (E133) and #2) dye per tablet until the coloration identical to the supplement was achieved.

Statistical analyses

Data were analyzed with JAMOVI software. The normality assumption was tested for using the Shapiro-Wilk test ($p = 0.736$). Paired samples t-tests were used to determine differences between the nitrate and placebo conditions in the total numbers of repetitions. Taking into account the trial order, an additional paired samples t-test was used to determine if a learning effect was present between the first (visit 1) and second sessions (visit 2), irrespective of treatment group (Stein et al., 2020). Descriptive data are provided as mean ± standard deviation. The magnitude of treatment effects (ES) were estimated with Cohen's D and classified as "trivial" (<0.19), "small" (0.20–0.49), "moderate" (0.50–0.79), and "large" (>0.80) (Sullivan & Feinn, 2012). The level of significance was set at $p < 0.05$.

Results

All the recruited participants successfully completed the trial, and their data were included in the analysis. Participants age ranged from 19 − 44 years and BMI 18.2 − 29.2 kg/m^2, with varying CrossFit® experience ranging between 10 months to 9 years (108 months) (Table 1).

Participants performed between 6 to 31 rounds (180 to 930 repetitions) upon consuming beetroot supplement, which was similar to the range of rounds (6 to 28 rounds; 180 to 840 repetitions) upon placebo intake (Table 1). The t-test did not show significant differences between beetroot supplement and placebo conditions for CrossFit® performance, considering the number of rounds of a Cindy workout for 20 minutes (17.4 ± 2.7 vs. 16.7± 2.3 rounds, $p= 0.078$). A significant learning effect was detected between the first and second sessions (16.6 ± 2.4 vs. 17.5 ± 2.6 rounds, $p= 0.034$). As seen in Figure 3, the means of both conditions were similar; the average of 0.7 more rounds completed (ES = 0.28; small effect) in the supplement condition was not statistically significant. A significant learning effect was observed, with higher performance in session 2 (Figure 4). No athlete reported any effect after the beetroot supplement intake. Otherwise, two participants (10 %) reported increased activeness after the placebo intake.

Table 1. Athlete's demographics.

Variables	Mean	SD
Age (years)	28	7
Weight (kg)	72.2	9.8
Height (cm)	170.9	4.5
BMI (kg/m^2)	24.7	3.2
CrossFit® Experience (months)	36.2	30.5

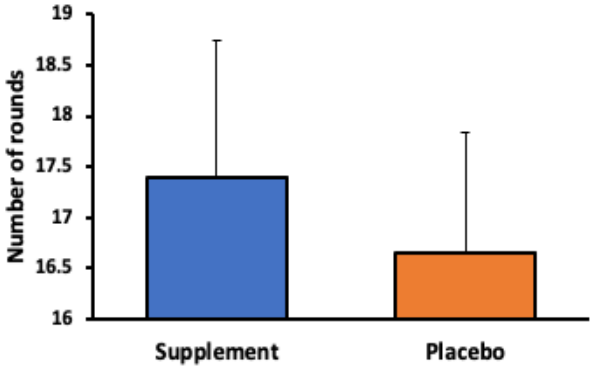

Figure 3. Number of rounds completed (mean ± standard deviation) by participants in Cindy protocol after consumption of supplement (beetroot powder) and placebo.

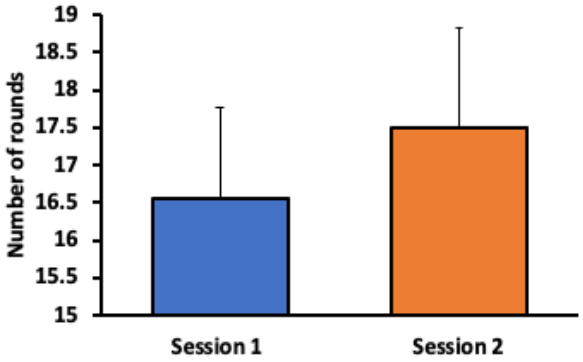

Figure 4. Number of rounds completed (mean ± standard deviation) by participants in Cindy protocol during the first session (testing visit 1) and second session (testing visit 2).

Discussion

This study examined differences in CrossFit® performance after acute nitrate supplementation compared to placebo among trained men. Contrary to our initial hypothesis, the results indicated no significant improvement in performance metrics, specifically the number of rounds completed in the "Cindy" protocol, following ingestion of beetroot powder compared to a placebo. The reason for this could be manifold including the type of supplement, dosing regime, and exercise performance metrics used in the current study.

In contrast to the current study, previous research has documented performance enhancements following beetroot supplementation in sporting activities with varying physiological demands (Alsharif et al., 2023; Coggan et al., 2021; Gao et al., 2021). While the dose used in the current study is consistent with current recommendations (8–16 mmol) (Shannon et al., 2022), it should be acknowledged that most prior research has utilised concentrated beetroot juice rather than tablets. Therefore, the different food matrices and processing methods of the supplements might account for the discrepancy between our findings and the findings of others. Moreover, only two other studies have investigated the acute effects of dietary nitrate on CrossFit® performance (Garnacho-Castano et al., 2020; Garnacho-Castano et al., 2022). Unlike the current study, Garnacho-Castaño et al. (2020) reported beetroot juice (~ 12.8 mmol of nitrate) improved the number of wall ball shots and full back squats completed with a 3-min rest, but not when no rest was given. These data suggest that nitrate supplementation could benefit CrossFit® requiring aerobic energy production, where enhanced blood flow and oxygen delivery play crucial roles. This is further supported by the reported reduced oxygen cost during the rest period and back squats with the beetroot (Garnacho-Castano et al., 2022). Apart from the higher dose (12.8 vs 8.3 mmol) that study also had a longer absorption period (3 vs 1.5 hours) as compared to our study which might account for the differences. With regards to the pharmacodynamics of

nitrate, after ingestion of beetroot plasma nitrate peaks 1-2 hours postprandial, whereas plasma nitrite peaks 2-3 hours as it is recycled to the mouth before conversion by nitrite-producing oral bacteria (Bryan et al., 2022; Wylie et al., 2016) . Given that nitrite is the precursor to NO, the absorption period in the current study was likely insufficient to allow the maximum physiological benefits.

Other than the type of supplement and dosing regime, the exercise protocol could potentially be a key factor affecting the supplementation efficacy investigations. Our study assessed CrossFit® performance via "Cindy", which has been widely adopted in research (Oliveira et al., 2023; Stein et al., 2020; Ziyaiyan et al., 2023), allowing for meaningful comparisons. In agreement with the current study, previous studies have found no effect of acute caffeine (Stein et al., 2020), caffeine with sodium bicarbonate (Ziyaiyan et al., 2023), and capsiate (Oliveira et al., 2023) supplementation on the "Cindy" protocol, despite the widely documented ergogenic effects. Thus, while "Cindy" represents an ecologically valid measurement of CrossFit® performance, it is possible that this performance metric is not sensitive enough to nutritional supplementation. Moreover, we found a significant learning effect between the first and second sessions, irrespective of the supplementation group, even though participants were familiar with CrossFit®. These findings are consistent with Stein et al. (2020) who also reported a learning effect, likely due to participants becoming more familiar with the specific demands of the CrossFit® workout used in the study. This learning effect is important to consider as it may have masked any subtle effects of beetroot supplementation. Future studies should consider the sensitivity of the performance measure and suitable familiarization protocols to eliminate potential learning effects so that evidence-based recommendations on supplement use for CrossFit® can be made.

This study is not without limitations, which should be considered when interpreting the findings. The detection of a learning effect suggests that the study design might have inadvertently introduced a variable that confounded the results. While this learning effect reflects natural improvements over time, it complicates the ability to isolate the effects of the beetroot supplementation. Additionally, the sample size, although adequate for the analysis, may not have been large enough to detect subtle effects of beetroot on performance. Furthermore, individual variability in response to beetroot supplementation, due to factors such as baseline training status, could have contributed to the null findings. Finally, the specific CrossFit® workout chosen for this study, while representative, might not encompass the full spectrum of CrossFit® activities, potentially limiting the generalizability of our results.

Conclusion

The findings of this study indicate that the intake of 515 mg (8.3 mmol) of beetroot nitrate, 90 minutes before a Cindy workout, does not improve CrossFit® performance. More studies are needed to confirm whether nitrate in beetroot can be used as an effective performance improvement strategy in CrossFit® male athletes since a learning effect was found for the workout Cindy. Future research should examine a learning effect in the practice of CrossFit® workouts.

Acknowledgements: The authors would like to thank the Cambridge University ReachSci Society for their support and assistance. This project was an outcome of the Mini-PhD Programme 2023: Food Science & Nutrition.

Conflict of interest: All authors declare that they have no conflict of interest relevant to the content of this article.

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