

Effect of Beetroot Juice Supplementation on Mood, Perceived Exertion, and Performance During a 30-Second Wingate Test

Pablo Jodra, Raúl Domínguez, Antonio J. Sánchez-Oliver, Pablo Veiga-Herreros, and Stephen J. Bailey

Purpose: Dietary supplementation with inorganic nitrate (NO_3^-) can enhance high-intensity exercise performance by improving skeletal muscle contractility and metabolism, but the extent to which this might be linked to altered psychophysiological processes is presently unclear. The purpose of this study was to assess the effects of NO_3^- -rich beetroot juice (BJ) supplementation on profile of mood states, ratings of perceived exertion (RPE), and performance in a 30-second Wingate cycle test. **Methods:** In a double-blind, randomized, cross-over study, 15 subjects completed 2 laboratory sessions after ingesting NO_3^- -rich or NO_3^- -depleted (placebo) BJ. Participants initially completed the profile of mood states questionnaire. Subsequently, participants completed a warm-up followed by a 30-second all-out Wingate cycling test. After the Wingate test, participants immediately indicated the RPE of their leg muscles (RPE_{muscular}), cardiovascular system (RPE_{cardio}), and general RPE (RPE_{general}). **Results:** Compared with the placebo condition, supplementation with BJ increased peak power output (W_{peak}) (+4.4%, 11.5 [0.7] vs 11.1 [1.0] W·kg⁻¹; P = .039) and lowered the time taken to reach W_{peak} (7.3 [0.9] vs 8.7 [1.5] s; P = .002) during the Wingate test. The profile of mood states score linked to tension was increased prior to the Wingate test (4.8 [3.0] vs 3.4 [2.4]; P = .040), and RPE_{muscular} was lowered immediately following the Wingate test (17.7 [1.6] vs 18.3 [1.0]; P = .031), after BJ compared with placebo ingestion. **Conclusions:** Acute BJ supplementation improved pre-exercise tension, 30-second Wingate test performance, and lowered postexercise RPE_{muscular}.

Keywords: dietary supplement, ergogenic aid, high-intensity exercise, nitrate, mood states

In high-performance sports, even a small increment in performance can have a large impact on competition outcome. With this in mind, many athletes attempt to boost their performance through the consumption of purported nutritional ergogenic aids. However, although many commercially available supplements claim to improve sports performance, such claims are not always supported by a firm foundation of robust scientific evidence. To overcome this ambiguity and to provide evidence-based recommendations for dietary supplementation to enhance sports performance, the International Olympic Committee has recently published a classification for nutritional supplements based on the scientific evidence to support their ergogenic efficacy. One dietary supplement classified as having a high level of scientific evidence to support an ergogenic effect was inorganic nitrate (NO₃⁻).

The ergogenic effects of NO₃⁻ supplementation, which is inically administered in the form of NO₃⁻-rich beetroot juice puently, nitric oxide (NO) as NO₃⁻ is considered biologically inert.² After NO₃⁻ supplementation, the increase in plasma [nitrite] serves as a circulating substrate for O₂-independent NO generation, with the reduction of nitrite to NO enhanced in conditions of hypoxia and acidosis.² Some of the physiological processes that have been reported to be enhanced by increased NO exposure,

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which might underpin the ergogenic effects of NO₃⁻ supplementation, include improvements in muscle vasodilation and blood flow,³ metabolic responses,⁴ and contractile force.⁵ Moreover, it is well documented that type II, fast-twitch skeletal muscle is more hypoxic³ and acidic⁶ compared with type I, slow-twitch muscle during contractions, and that type II muscle is heavily recruited during high-intensity intermittent and all-out sprint exercise.⁷ Therefore, the potential for an ergogenic effect following dietary NO₃⁻ supplementation might be greatest during short-duration, high-intensity exercise, as supported by improved single sprint and/or repeated sprint/high-intensity intermittent exercise performance following NO₃⁻ supplementation.⁸

In addition to physiological factors within the skeletal muscle, it is recognized that psychological factors, such as mood and ratings of perceived exertion (RPE), can play a role in determining exercise performance.9 There is some evidence to suggest that ergogenic supplements, when consumed in large amounts, may raise levels of subjective tension, 10 which is considered the internal sensation of preparation to react immediately and with sufficient intensity to a demanding task.¹¹ However, the general consensus is that psychophysiological activation leads to improved performance up to a certain tension threshold, with diminished performance manifesting above this critical threshold.¹² In addition, subjective RPE, which characterizes the combination of feelings related to the execution of a physical exercise task, and has been considered to reflect the integrated response of feedback from central, peripheral, and metabolic factors, 13 has been reported to exhibit a positive correlation with objective physiological indicators. However, the effect of NO₃ supplementation on RPE is equivocal, 14,15 and no investigation has partitioned the effect of NO₃⁻ supplementation on general, cardiovascular and muscular RPE. Moreover, the effect of NO₃⁻ supplementation on other

psychological components, such as mood, is undefined, despite some evidence that NO₃⁻ intake can improve brain blood flow, ¹⁶ which is an important determinant of RPE and mood state profile. ¹⁷

The purpose of this study was to assess the effects of NO₃⁻ supplementation on RPE, mood profile, and performance in a 30-second Wingate cycle test in resistance-trained males. Resistance-trained participants were selected on the basis that resistance training elicits greater skeletal muscle hypertrophy in type II compared with type I muscle fibers such that a greater portion of muscle volume is likely composed of type II muscle in resistance-trained participants.¹⁸ Therefore, resistance-trained participants might be particularly well placed to exhibit improved sprint performance following NO₃⁻ supplementation. It was hypothesized that, compared with a NO₃⁻-depleted BJ placebo, a NO₃⁻-rich BJ would improve mood, lower RPE, and enhance 30-second Wingate cycle test performance.

Materials and Methods

Study Participants

Fifteen resistance-trained, male undergraduate students participated in this study (see Table 1 for the participant characteristics). Participation was voluntary after prospective participants met the following inclusion criteria, which were established in a preliminary prescreening session: (1) having completed at least 3 sessions per week of strength training within the past 18 months; (2) a bench press 1-repetition maximum greater than body mass and full-squat 1-repetition maximum at least 1.5 times greater than body mass; (3) no nutritional supplements had been consumed for at least 3 months before the study onset; (4) nonsmoker; (5) no cardiovascular, respiratory, metabolic, neurological, or orthopedic disorders that could interfere with cycle ergometer performance; (6) not a full-time professional athlete; and (7) experience with the Wingate test, having performed at least one test in the 3 months preceding the study commencement. Moreover, the investigators informed the participants of the study goals and test protocols, including the dietary requirements to be followed and the avoidance of other dietary supplements, during this prescreening session. After participants met the inclusion criteria and agreed to participate, they provided their written informed consent to participate in the study. The study protocol was approved by the ethics committee of the Universidad Alfonso X El Sabio (Madrid, Spain).

Study Design

The study adopted a randomized, double-blind, cross-over experimental design. Each participant completed 2 test sessions separated by 72 hours in an exercise physiology laboratory at the same time of day (± 0.5 h). Subjects were instructed to refrain from any type of physical exercise for 72 hours before the first session until the end

Table 1 Participant Characteristics

Variable	Value		
Age, y	23 (2)		
Height, m	1.78 (0.06)		
Weight, kg	75.6 (8.9)		
Body mass index, kg/m ²	23.9 (2.1)		

Note: Data are expressed as mean (SD).

of the study. All subjects were given strict guidelines to ensure their diet comprised a similar macronutrient composition (60% carbohydrates, 30% lipids, and 10% proteins) during the investigation. Participants were instructed to record their food intake for 48 hours prior to the first supplementation trial and to reproduce this prior to the second supplementation trial. Upon arrival at the laboratory for each session, participants ingested BJ or placebo. In the first experimental session, 8 subjects ingested BJ supplementation and 7 subjects ingested placebo. One hundred and fifty minutes after ingesting the supplement, participants completed the profile of mood states (POMS) questionnaire. Participants then completed a 30-second Wingate test on a cycle ergometer after a warm-up. Immediately after the test, participants graded their exertion using the RPE scale (see Figure 1).

BJ Supplementation. Participants arrived at the laboratory 3 hours before initiating the Wingate test. Upon arrival, participants consumed a 70-mL BJ supplement that was either enriched in NO₃⁻ (\sim 6.4 mmol of NO₃⁻) or depleted in NO₃⁻ (0.04 mmol of NO₃⁻) as placebo (Beet It; James White Drinks Ltd, Ipswich, UK). The timing of BJ ingestion was based on the recommendation of ingesting the supplement 2.5 to 3 hours before starting an exercise effort to coincide with peak plasma [NO₂⁻]. To avoid a potential confounding influence from habitual dietary NO₃⁻ intake, subjects were given a list of foods high in NO₃⁻ to avoid for 48 hours before each session. Furthermore, to control for a possible ergogenic effect of caffeine ingestion on test performance, the intake of caffeine was also restricted 24 hours before the study start, and the subjects were provided with a list of foodstuffs rich in caffeine to avoid. Finally, subjects were instructed to avoid brushing their teeth on the morning of testing and the use of antibacterial mouthwash, which would alter the oral microbiota and interfere with NO₃⁻ reduction, from 1 week prior to the first laboratory visit and for the duration of the study.

Profile of Mood States. To assess the participants' mood, the POMS questionnaire was used in its original reduced version, which has been translated into Spanish and validated by Fuentes et al. ¹⁹ Participants graded a set of 29 adjectives related to 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 6 dimensions: tension (T), depression (D), anger (A), vigor (V), fatigue (F), and confusion (C).

Wingate Test. The Wingate test was completed on a cycle ergometer (Ergomedic 828E; Monark Exercise, Vansbro, Sweden). Prior to completing the Wingate test, participants performed a 5-minute warm-up at a self-selected submaximal cycling workload. After 1 minute of passive rest, subjects subsequently completed a specific warm-up comprising 3 minutes of cycle exercise at 120 W (60 rpm), with a maximum 5-second sprint completed at the end of each minute. After 2 minutes of rest, the Wingate test was completed.

The Wingate test consisted of 30-second maximal cycling, commenced with the pedals stationary, with the resistance on the flywheel set to 7.5% of the participant's body mass. Participants were instructed to (1) commence the first pedal stroke with the dominant leg, (2) reach the maximum rpm in the shortest time possible, and (3) provide a maximal effort to maintain this pedaling speed until the end of the test. Power output was recorded during each second of the test. The following variables were subsequently calculated: peak power $(W_{\rm peak})$, the time to reach $W_{\rm peak}$ (time-to- $W_{\rm peak}$), mean W for the test duration $(W_{\rm mean})$, and minimum power $(W_{\rm min})$, taken as the lowest W recorded during the last 10 seconds of the test.



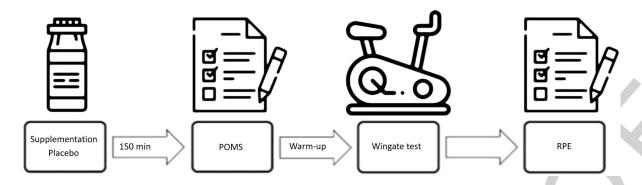


Figure 1 — Flow diagram showing the different stages of each experimental session. POMS indicates profile of mood states; RPE, rating of perceived exertion.

tings of Perceived Exertion. As soon as participants had appleted the Wingate test, they were presented with the 6 to 20 RPE scale. Participants were then asked to indicate the RPE related to their leg muscles (RPE_{muscular}), cardiovascular system (RPE_{cardio}), and general overall RPE (RPE_{general}).

Statistical Analysis

All data were initially tested for normal distribution using Shapiro–Wilk tests. Subsequently, Student t tests were used to compare the outcome variables between the 2 experimental conditions (placebo and BJ). The Wilcoxon test was used for data that were not normally distributed (RPE_{muscular} and RPE_{general}). Effect size (ES) was calculated using Cohen d with values of <0.2, 0.5 to 0.8, and >0.8 reflective of trivial, moderate, and large ESs, respectively. All data are reported as mean (SD). Statistical significance was set at P < .05. Statistical tests were performed using SPSS (version 18.0; SPSS, Chicago, IL).

Results

The $W_{\rm peak}$, time-to- $W_{\rm peak}$, $W_{\rm mean}$, and $W_{\rm min}$ variables are illustrated for a representative individual in Figure 2 and presented as group mean values in Figure 3. The $W_{\rm peak}$ was higher (+4.4%, 11.5 [0.7] vs 11.1 [1.0] W/kg; t=-2.280, ES = 0.48, P=.039; Figure 3), and time-to- $W_{\rm peak}$ was lower (7.3 [0.9] vs 8.7 [1.5] s; t=3.898, ES = 1.17, P=.002; Figure 2) after BJ compared with placebo

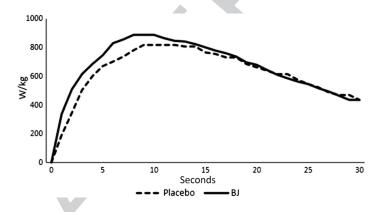


Figure 2 — Power output (W/kg) profile during the 30-second Wingate test for a representative subject in the placebo and BJ conditions. BJ indicates beetroot juice.

ingestion. There were no differences in $W_{\rm mean}$ and $W_{\rm min}$ between the BJ and placebo conditions ($W_{\rm mean}$: 8.6 [0.6] vs 8.5 [0.8] W/kg; t=-1.379, ES = 0.19, P=.104 and $W_{\rm min}$: 6.2 [0.8] vs 6.0 [0.9] W/kg; t=-1.064, ES = 0.24, P=.305; Figure 3).

For the different POMS dimensions, a higher tension score was reported in the BJ condition (4.80 [2.98]) compared with the placebo condition (3.40 [2.38]; ES = 0.53, P = .040). No significant differences between the 2 experimental conditions were detected in any of the other POMS dimensions (Table 2).

Immediately following the Wingate test, RPE_{muscular} was lower in the BJ compared with the placebo condition (17.7 [1.6] vs 18.3 [1.0]; z=-2.157, ES = 0.47, P=.031). There were no differences in RPE_{cardio} and RPE_{general} between the BJ and placebo conditions (RPE_{cardio} = 17.4 [1.6] vs 17.7 [1.63]; t=0.521, ES = 0.19, P=.610 and RPE_{general} = 18.1 [1.3] vs 18.3 [0.9]; z=0.926, ES = 0.19, P=.334; Table 3).

Discussion

The principal novel findings of the current study were that acute BJ supplementation increased tension rating prior to, improved performance during, and lowered muscle RPE immediately following a 30-second Wingate test in resistance training participants. These findings are consistent with our experimental hypotheses and

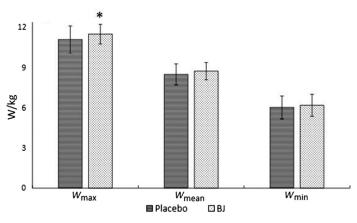


Figure 3 — Power output (W/kg) variables during the Wingate test in the placebo and BJ supplementation conditions. Data are expressed as mean (SD). BJ indicates beetroot juice; W_{mean} , mean power output; W_{min} , minimum power output; W_{peak} , peak power. *Significantly greater than placebo (P < .05).

Table 2 Scores Reported for the Different Dimensions of the POMS Questionnaire in the 2 Experimental Conditions (Placebo and Beetroot Juice)

Dimension	Placebo	Beetroot juice	ES	Р
Tension	3.4 (2.4)	4.8 (3.0)	0.53	.040*
Depression	2.3 (3.6)	1.4 (1.4)	0.34	.551
Anger	2.4 (3.7)	1.2 (2.6)	0.39	.233
Vigor	12.5 (3.1)	13.3 (3.3)	0.26	.464
Fatigue	3.7 (3.9)	3.1 (2.6)	0.19	.916
Confusion	13.6 (2.4)	13.5 (2.4)	0.04	.926

Abbreviations: ES, effect size; POMS, profile of mood states. Note: Data are expressed as mean (SD).

Table 3 Scores Reported for RPE in the 2 Experimental Conditions (Placebo and Beetroot Juice)

Dimension	Placebo	Beetroot juice	ES	P
RPE _{muscular}	18.3 (1.0)	17.7 (1.6)	0.47	.031*
RPE _{cardio}	17.7 (1.6)	17.4 (1.6)	0.19	.610
$RPE_{general}$	18.3 (0.9)	18.1 (1.3)	0.19	.334

Abbreviations: ES, effect size; RPE, rating of perceived exertion. Note: Data are expressed as mean (SD).

suggest that improvements in psychophysiological processes might also contribute to the ergogenic effects of NO₃⁻ supplementation during short-duration, high-intensity exercise.

In the present study, participants consumed 70 mL of BJ or placebo providing ~6.4 and 0.04 mmol of NO_3^- , respectively. This dose of NO_3^- has been reported to increase plasma [nitrite] in numerous studies, 20 with the subsequent reduction of circulating nitrite to NO believed to underpin the ergogenic effects of NO_3^- supplementation. Therefore, although plasma [nitrite] was not assessed in the present study, we adopted a protocol that has been consistently shown to enhance plasma [nitrite] by a magnitude that would be expected to improve exercise performance. Indeed, acute supplementation with a similar dose of NO_3^- has previously been reported to enhance performance.

The acute ingestion of BJ increased $W_{\rm peak}$ compared with the placebo condition (+4.4%), consistent with some previous studies reporting increased $W_{\rm peak}$ (~+6%) in the Wingate test²² and other protocols designed to assess $W_{\rm peak}$ on a cycle ergometer.²³ In addition to this improvement in $W_{\rm peak}$, we observed a reduction in the time-to- $W_{\rm peak}$ following BJ ingestion. This improvement in time-to- $W_{\rm peak}$ is also in line with a previous observation²⁴ following BJ consumption. As a greater and more rapid attainment of $W_{\rm peak}$ is an important determinant of performance in team sports and sprint events in athletics, track cycling, or speed skating, our findings might have implications for improving performance in these sports. Indeed, NO_3^- supplementation has been reported to improve performance during single sprints and repeated bouts of high-intensity exercise in certain experimental conditions. ^{15,24}

The improvements in $W_{\rm peak}$ and time-to- $W_{\rm peak}$ in the present study were accompanied by modifications to some factors that define an individual's mood state, as assessed via the POMS questionnaire. Specifically, compared with placebo, supplementation with BJ increased tension ratings. Although some studies have observed an increased tension rating concomitant with compromised exercise performance following caffeine supplementation, ²⁵ there is also evidence to suggest that increased tension reflects an optimal state of emotional preparation to undertake a physical task.

Indeed, tension has been suggested to exhibit a parabolic relationship with exercise performance whereby levels of tension that are too low or too high will elicit suboptimal performance. Therefore, our findings of improved $W_{\rm peak}$ and time-to- $W_{\rm peak}$ accompanied by increased tension following BJ supplementation are consistent with the notion that a small elevation in tension can enhance exercise performance. This potential for increased tension to have enhanced performance in this study might be linked to an increased willingness to commit to the task and/or greater sensations of alertness and optimism when facing the competitive situation.

Several studies have assessed the effect of BJ supplementation on RPE. Although some authors have reported improved performance without any significant impact on RPE, ¹⁴ others have shown performance improvements concomitant with a drop in RPE. ¹⁵ However, a novel contribution of our study was that participants were asked to provide specific RPE scores for RPE_{general}, RPE_{muscular}, and RPE_{cardio} as opposed to just RPE_{general}, which has been assessed in previous studies. ^{14,15} Our finding that BJ supplementation selectively lowered RPE_{muscular}, but not RPE_{general} or RPE_{cardio}, provides novel information pertaining to the potential mechanism for the ergogenic effect of BJ supplementation.

Among the possible mechanisms that could explain the effects of BJ on RPE is an enhancement in blood flow to the frontal lobe of the brain. 16 This brain region processes emotions and decision making and regulates motor control, 27 which all contribute to the integrative subjective perception of exertion. 17 In addition to influencing mood, brain blood flow and oxygenation have important implications for exercise performance. Indeed, reduced blood flow to the brain during an exercise effort has been identified as a factor promoting the onset of fatigue. 28 Therefore, enhanced brain blood flow could have contributed to the lower RPE_{muscular} and improved performance after BJ supplementation in the current study. Alternatively, or in conjunction with enhanced brain perfusion, the lowering in RPE_{muscular}, but not RPE_{general} or RPE_{cardio}, after BJ supplementation could be linked to a lower muscle metabolic perturbation 4 and a subsequent reduction in type III/IV



^{*}Significant difference between the placebo and beetroot juice conditions (P < .05).

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skeletal muscle afferent feedback.²⁹ Further research is required to resolve the underlying mechanisms for the lower RPE_{muscular} after BJ supplementation and its relative importance to the ergogenic effects of this nutritional intervention.

Practical Applications

In the present study, acute BJ ingestion increased W_{peak} and lowered the time to achieve this higher W_{peak} , without altering the W_{mean} , compared with the placebo condition. This improvement in variables related to acceleration and peak power output after BJ supplementation might be expected to translate into enhanced acceleration, rate of force development, and peak force development. Subsequently, these enhancements might be expected to improved performance in sports, such as sprinting events in track-and-field athletics, track cycling and speed skating, or powerlifting, where acceleration, rate of force development, and peak force development are key performance determinants. However, further research is required to determine whether the findings from the current study can be reproduced in field settings. It should also be acknowledged that, as subjects were required to abstain from foods rich in NO₃⁻ for the duration of this study, this could have contributed to the positive effects of BJ supplementation in the current study.

Conclusions

The acute ingestion of BJ in resistance-trained athletes improved performance in a 30-second Wingate test, as evidenced by an increased peak power and the shortened time to reach this maximum power. This improvement in Wingate test performance was accompanied by an increased feeling of tension prior to the test and a lower RPE of the leg muscles immediately following the test in the BJ condition compared with the placebo condition. These novel observations suggest that BJ supplementation might have implications for improving performance in speed/power athletes and that this ergogenic effect might be linked, at least in part, to an improvement in psychophysiological processes.

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Queries

- Q1. Please note that the phrase "which is typically administered in" in sentence starting with "The ergogenic effects" is not grammatically correct in this context (The ergogenic effects are not "typically administered"). Suggest separating for clarity: "NO₃⁻ supplementation is typically administered in the form of NO₃⁻-rich beetroot juice (BJ). The ergogenic effects of NO₃⁻ supplementation are attributed to
- Q2. Please ensure author information is listed correctly here and within the byline.
- Q3. Please check if thr edits to the sentence "The Wingate test was . . . " are correct and convey the intended meaning.
- Q4. Please check and confirm the hierarchy of head levels throughout the article.
- Q5. As per journal style, "mean ± SD" should be represented as "mean (SD)." Hence, the values are changed accordingly. Please check and confirm.
- **Q6.** Originally, Refs. 4 and 29 were identical. Hence the duplicate has been removed from the reference list and the subsequent references have been renumbered both in text and in reference list. Please verify.
- Q7. Please provide the conference date (ie, day and month) and conference location for Kef 18.