Effect of Beetroot Juice Supplementation on Mood, Perceived Exertion and Performance during a 30 s Wingate Test

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Abstract

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 (POMS), ratings of perceived exertion (RPE) and performance in a 30 s Wingate cycle test. **Methods:** In a double blind, randomized, crossover study, 15 subjects completed two laboratory sessions after ingesting NO_3^--rich or NO_3^--depleted (placebo) BJ. Participants initially completed the POMS questionnaire. Subsequently, participants completed a warm-up followed by a 30 s 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 to the placebo condition, supplementation with BJ increased peak power output (W_{peak}) (+4.4%, 11.5 ± 0.7 vs. 11.1 ± 1.0 $W \cdot kg^{-1}$, p = 0.039) and lowered the time taken to reach W_{peak} (7.3 ± 0.9 vs. 8.7 ± 1.5 s, p = 0.002) during the Wingate test. The POMS score linked to tension was increased prior to the Wingate test (4.8 ± 3.0 vs. 3.4 ± 2.4, p = 0.040), and $RPE_{muscular}$ was lowered immediately following the Wingate test (17.7 ± 1.6 vs. 18.3 ± 1.0, p = 0.031), after BJ compared to placebo ingestion. **Conclusions:** Acute BJ supplementation improved pre-exercise tension, ~~and~~ 30 s Wingate test performance, and lowered post-exercise $RPE_{muscular}$.

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

Introduction

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 [1]. One dietary supplement classified as having a high level of scientific evidence to support an ergogenic effect was inorganic nitrate (NO_3^-).

The ergogenic effects of NO_3^- supplementation, which is typically administered in the form of NO_3^--rich beetroot juice (BJ), is attributed to its stepwise reduction to nitrite and subsequently nitric oxide (NO) as NO_3^- is considered biologically inert [2]. After NO_3^- supplementation, the increase in plasma [nitrite] serves as a circulating substrate for O_2-independent NO generation [2], with the reduction of nitrite to NO enhanced in conditions of hypoxia and acidosis [2,3]. Some of the physiological processes that have been reported to be enhanced by increase NO exposure, which might underpin the ergogenic effects of NO_3^- 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 hypoxic [3] and acidic [6] compared to type I slow-twitch muscle during contractions [4] 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 NO_3^-

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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 NO_3^- 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 [11,8]. However, the general consensus is that psychophysiological activation leads to improved performance up to a certain tension threshold, with diminished performance manifest 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 [11,7]. However, the effect of NO_3^- supplementation on RPE is equivocal [14,15], and no investigation has partitioned the effect of NO_3^- supplementation on general, cardiovascular and muscular RPE. Moreover, the effect of NO_3^- supplementation on other psychological components such as mood is undefined, despite some evidence that NO_3^- intake can improve brain blood flow [16] which is an important determinant of RPE and mood state profile [27].

The purpose of this study was to assess the effects of NO_3^- supplementation on RPE, mood profile and performance in a 30 s 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 to type I muscle fibers such that a greater portion of muscle volume is likely comprised of type II muscle in resistance trained participants [17]. Therefore, resistance trained participants might be particularly well placed to exhibit improved sprint performance following NO_3^- supplementation. It was hypothesized that, compared to a NO_3^--depleted BJ placebo, NO_3^--rich BJ would improve mood, lower RPE and enhance 30 s Wingate cycle test performance.

Materials and Methods

Study participants

The study participants were 15 resistance trained male undergraduate students (see Table 1 for the participant characteristics). Participation was voluntary after prospective participants met the following inclusion criteria, which were established in a preliminary pre-screening session: a) having completed at least 3 sessions per week of strength training within the past 18 months; b) a bench press one-repetition maximum (1 RM) greater than body mass and full squat 1 RM -at least 1.5 times greater than body mass; c) no nutritional supplements had been consumed for at least three months before the study onset; d) non-smoker; e) no cardiovascular, respiratory, metabolic, neurological or orthopedic disorders that could interfere with cycle ergometer performance; f) not a full-time professional athlete; and g) experience with the Wingate test, having performed at least one test in the 3 months preceding the study commencement. Moreover, three 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 pre-screening session. After participants met the inclusion criteria and agreed to participate, they provided their

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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, crossover experimental design. Each participant completed two test sessions separated by 72 h 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 from 72 hours before the first session until the end 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 s 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).

Beetroot juice supplementation

Participants arrived at the laboratory 3 h before initiating the Wingate test. Upon arrival, participants consumed a 70 ml BJ supplement that was either enriched in NO_3^- (~6.4 mmol NO_3^-) or depleted in NO_3^- as placebo (0.04 mmol NO_3^-) (Beet It; James White Drinks Ltd, Ipswich, UK). The timing of BJ ingestion was based on the recommendation of ingesting the supplement 2.5-3 h before starting an exercise effort to coincide with peak plasma [NO_2^-]. To avoid a potential confounding influence from habitual dietary NO_3^- intake, subjects were given a list of foods high in NO_3^- to avoid for 48 h before each session. Further, to control for a possible ergogenic effect of caffeine ingestion on test performance, the intake of caffeine was also restricted 24 h 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 use of antibacterial mouthwash, which would alter the oral microbiota and interfere with NO_3^- reduction, from one week prior to the first laboratory visit and for the duration of the study.

Profile of mood states (POMS)

To assess the participants' mood, the profile of mood states (POMS) questionnaire was used in its original reduced version[14], which has been translated into Spanish and validated by Fuentes et al. [18](1995). 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 six dimensions: tension (T), depression (D), anger (A), vigor (V), fatigue (F) and confusion (C).

Wingate test

The Wingate test was completed on a Monark cycle ergometer (Ergomedic 828E, Vansbro, Sweden). Prior to completing the Wingate test, participants performed a 5 min warm up at a self-selected submaximal cycling workload. After 1 min of passive rest, subjects subsequently completed a specific warm up comprising 3 min of cycle exercise

Human Kinetics, 1607 N Market St, Champaign, IL 61825

at 120 W (60 rpm) with a maximum 5 s sprint completed at the end of each minute. After 2 min of rest, the Wingate test was completed.

The Wingate test consisted of 30 s maximal cycling, commenced with the pedals stationary~~starting from standstill~~, with the resistance on the flywheel set to 7.5% of the participant's body mass. Participants were instructed to: i) commence the first pedal stroke with the dominant leg; ii) reach the maximum rpm in the shortest time possible; and iii) ~~try~~ 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_{peak}), the time to reach W_{peak} (time-to-W_{peak}), mean W for the test duration (W_{mean}) and minimum power (W_{min}), taken as the lowest W recorded during the last 10 s of the test.

Ratings of perceived exertion (RPE)

As soon as participants had completed the Wingate test, they were presented with the 6-20 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's *t*-tests were used to compare the outcome variables between the two experimental conditions (placebo and BJ). The Wilcoxon test was used for data which were not normally distributed ($RPE_{muscular}$ and $RPE_{general}$).– Effect size was calculated using Cohen´s d with values of <0.2, 0.5-0.8 and >0.8 reflective of trivial, moderate and large effects sizes, respectively. All data are reported as mean ±~~and~~ standard deviation ~~(*M* ± *SD*)~~. Statistical significance was set at $p < 0.05$. Statistical tests were performed using the software package SPSS version 18.0 (SPSS, Chicago, III).

Results

The W_{peak}, time-to-W_{peak}, W_{mean} and W_{min} variables are illustrated for a representative individual in figure 2 and presented as group mean values in figure 3. The W_{peak} was higher (+4.4%, 11.5 ± 0.7 vs. 11.1 ± 1.0 W/kg, t = -2.280, ES = 0.48, p = *0.039*, figure 3), and time-to-W_{peak} was lower (7.3 ± 0.9 vs. 8.7 ± 1.5 s, t =3.898, ES = 1.17, p = *0.002*, Figure 2) after BJ compared to placebo ingestion. There were no differences in W_{mean} and W_{min} between the BJ and placebo conditions (W_{mean}, 8.6 ± 0.6 vs. 8.5 ± 0.8 W/kg, t = -1.379, ES = 0.19, p = 0.104; W_{min}, 6.2 ± 0.8 vs. 6.0 ± 0.9 W/kg, t = -1.064, ES = 0.24, p = 0.305, Figure 3).

For the different POMS dimensions, a higher tension score was reported in the BJ condition (4.80 ± 2.98) compared to the placebo condition (3.40 ± 2.38, ES = 0.53, p = 0.040). No significant differences between the two 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 to the placebo condition (17.7 ± 1.6 vs. 18.3 ± 1.0, z = -2.157, ES = 0.47, p = 0.031). 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 = 0.610; $RPE_{general}$ = 18.1 ± 1.3, vs. 18.3 ± 0.9, z = 0.926, ES = 0.19, p = 0.334) (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 s Wingate test in resistance training participants. These findings are consistent with our experimental hypotheses and suggest that improvements in psychophysiological processes might also contribute to the ergogenic effects of NO_3^- 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 [19], with the subsequent reduction of circulating nitrite to NO believed to underpin the ergogenic effects of NO_3^- supplementation [22]. Therefore, while 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 [20].

The acute ingestion of BJ increased W_{peak} compared with the placebo condition (+4.4%), consistent with some previous studies reporting increased W_{peak} (~ +6%) in the Wingate test [21] and other cycling protocols designed to assess W_{peak} on a cycle ergometer [22]. In addition to this improvement in W_{peak}, we also observed a reduction in the time-to-W_{peak} following BJ ingestion. This improvement in time-to-W_{peak} is also in line with a previous observation [23] following BJ consumption. Since a greater and more rapid attainment of W_{peak} is 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 [15,23].

The improvements in W_{peak} and time-to-W_{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 [24], 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 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_{peak} and time-to-W_{peak} accompanied by increased tension following BJ supplementation are consistent with the notion that a small elevation in tension can enhance exercise performance [25]. 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. While some authors have reported improved performance without any significant impact on RPE [14], others have been able to shown performance improvements concomitant with a drop in the RPE [15]. However, a novel contribution of our study was that participants were asked to provide specific RPE scores for general overall exertion ($RPE_{general}$), leg muscles ($RPE_{muscular}$) and the cardiovascular system (RPE_{cardio}) as opposed to just $RPE_{general}$ that has been assessed in previous studies [14,15,12,13]. 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,–[26]; and regulates motor control [26], which all contribute to the integrative subjective perception of exertion [27]. 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 [29] and a subsequent reduction in type III/IV skeletal muscle afferent feedback [30]. 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 to 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, since subjects were required to abstain from foods rich in NO_3^- 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 s Wingate test, as evidenced by an increased peak power and 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 to 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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Figure 1. Flow diagram showing the different stages of each experimental session.

Figure 2. Power output (W/kg) profile during the 30 s Wingate test for a representative subject in the placebo and BJ conditions.

Figure 3. Power output (W/kg) variables during the ~~30 s~~ Wingate test in the placebo and BJ supplementation conditions. Data are expressed as mean ± standard deviation~~M ± SD~~. Peak power, W_{peak}; mean power output, $W_{mean;}$ and minimum power output, W_{min}. * = significantly greater than placebo ($p < 0.05$).

Table 1 Participant characteristics

Variable	Value
Age (years)	23 ± 2
Height (m)	1.78 ± 0.06
Weight (kg)	75.6 ± 8.9
BMI (kg/m^2)	23.9 ± 2.1

Data expressed as ~~M~~ mean ± ~~SD~~standard deviation. BMI = body mass index

Table 2 Scores reported for the different dimensions of the POMS questionnaire in the two experimental conditions (placebo and beetroot juice)

Dimension	Placebo	Beetroot juice	ES	*p*
Tension	3.4 ± 2.4	4.8 ± 3.0	0.53	0.040*
Depression	2.3 ± 3.6	1.4 ± 1.4	0.34	0.551
Anger	2.4 ± 3.7	1.2 ± 2.6	0.39	0.233
Vigor	12.5 ± 3.1	13.3 ± 3.3	0.26	0.464
Fatigue	3.7 ± 3.9	3.1 ± 2.6	0.19	0.916
Confusion	13.6 ± 2.4	13.5 ± 2.4	0.04	0.926

Data expressed as mean ± standard deviation~~M ±SD~~. *Significant difference between the placebo and beetroot juice conditions ($p < 0.05$).

Table 3 Scores reported for RPE in the two experimental conditions (placebo and beetroot juice)

Dimension	Placebo	Beetroot juice	ES	*p*
$RPE_{muscular}$	18.3 ± 1.0	17.7 ± 1.6	0.47	0.031*
RPE_{cardio}	17.7 ± 1.6	17.4 ± 1.6	0.19	0.610
$RPE_{general}$	18.3 ± 0.9	18.1 ± 1.3	0.19	0.334

Data expressed as mean ± standard deviation~~M ±SD~~. *Significant difference between the placebo and beetroot juice conditions ($p < 0.05$).

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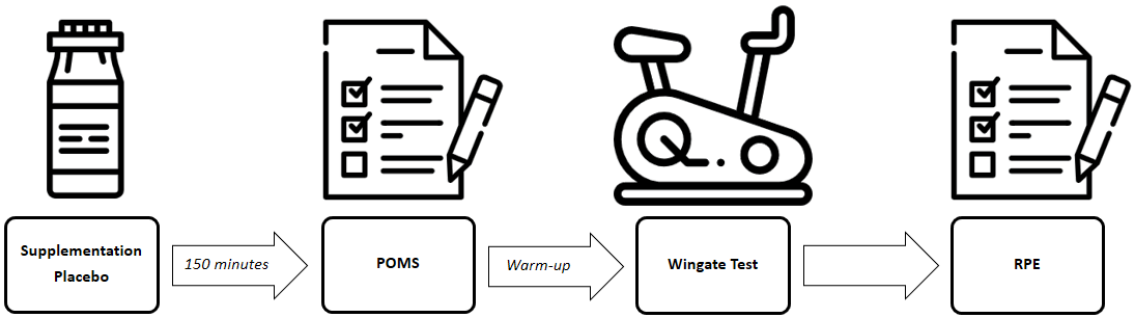

Figure 1. Flow diagram showing the different stages of each experimental session.

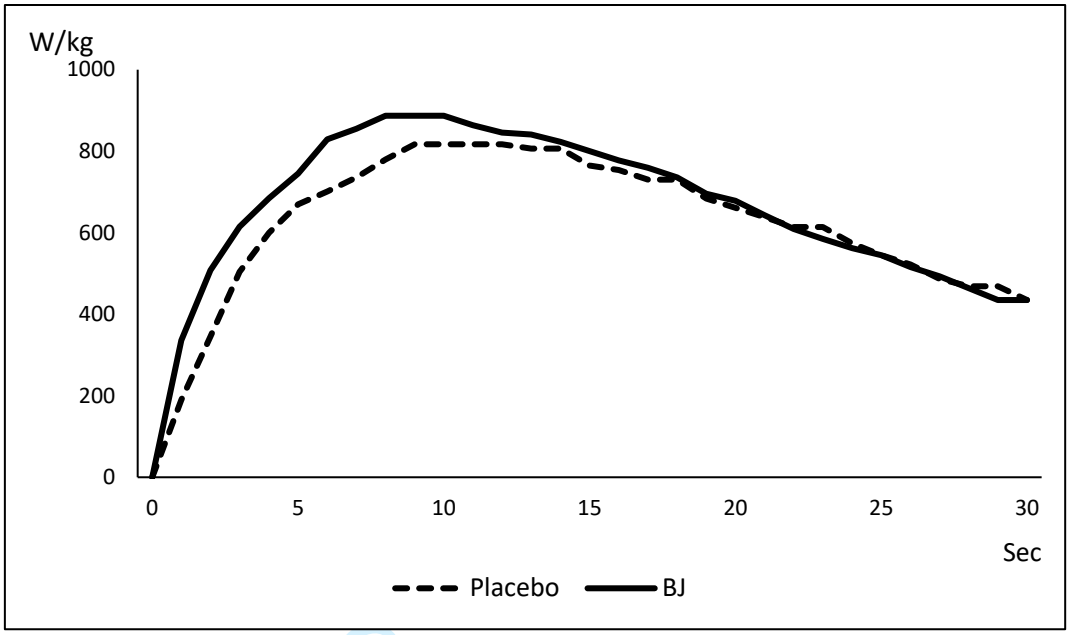

Figure 2. Power output (W/k) profile during the 30 sWingate test for a representative subject in the placebo and BJ conditions.

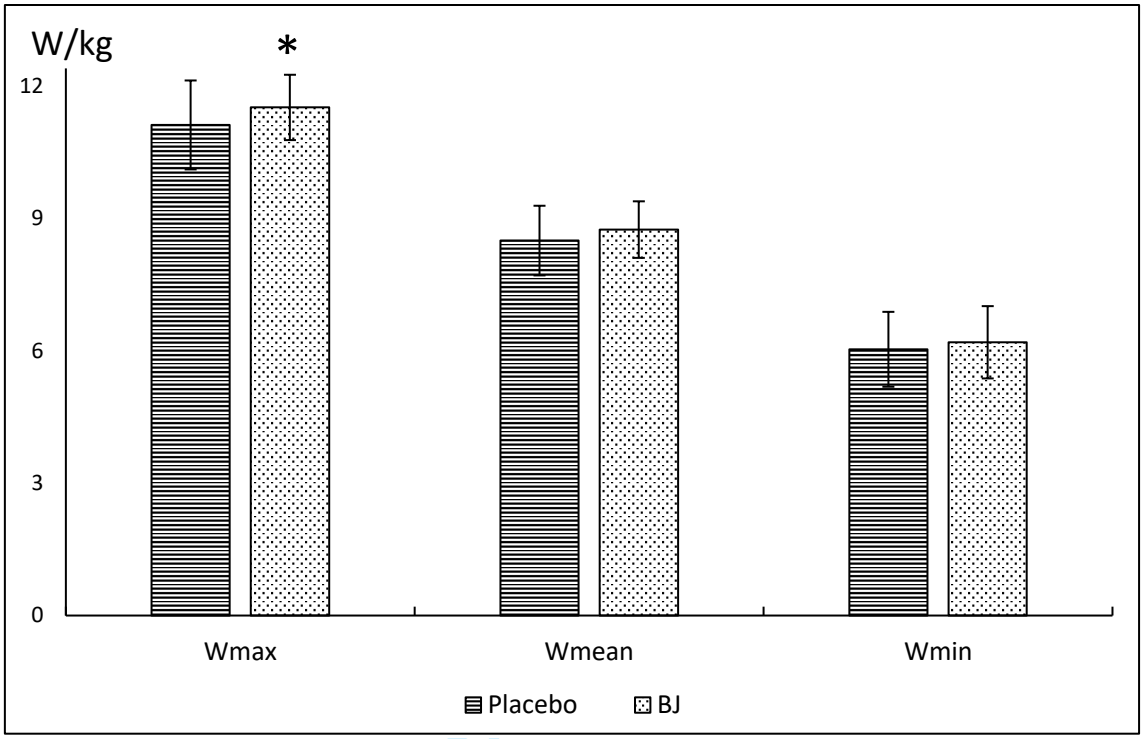

Figure 3. Power output (W/kg) variables, W_{peak}, W_{mean} and W_{min}, during the 30 s Wingate test in the placebo and BJ supplementation conditions.