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Acute Beet Juice Supplementation Does Not Improve 30- or 60-second Maximal Intensity Performance in Anaerobically Trained Athletes

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ABSTRACT

International Journal of Exercise Science 14(2): 60-75, 2021. Research suggests that beet juice is beneficial during aerobic exercise. However, the impact of beet juice during primarily anaerobic exercise is equivocal. The purpose of this study was to determine the effects of acute beet juice supplementation on maximal intensity performance during 30-s and 60-s maximal-intensity cycling sprints. Using a double-blind, crossover-study design, 14 anaerobically trained male hockey players completed six Wingate cycling tests: familiarization trials of a 30-s and 60-s Wingate test, followed by 30-s Wingate placebo/beet juice trials, and 60-s Wingate placebo/beet juice trials. Repeated measures ANOVAs were used to compare the change in power between conditions over the duration of each trial. Paired t-tests were run to compare performance between conditions of various work and power variables. One-way ANOVAs were utilized to compare the change between conditions of the 30-s test to the change between conditions of the 60-s test. Beet juice supplementation yielded no statistical differences from placebo in any of the measured variables during the 30-s or 60-s tests ($p > 0.05$). The percent change for fatigue index was significantly different between the 30-s and 60-s tests ($p = 0.032$) suggesting less fatigue with beet juice supplementation. Overall, beet juice did not improve maximal intensity performance during 30-s or 60-s cycling sprint tests. Performance during the 60-s bout was not impacted to a greater extent than the 30-s bout after beet juice supplementation. These results suggest that beet juice supplementation does not improve short-duration exercise performance in anaerobically trained athletes.

Key words: beetroot juice, dietary nitrate, power, sprint, Wingate

INTRODUCTION

In recent years, research on exogenous dietary nitrate (NO_3-) supplementation during exercise has seen a dramatic increase (26). Most of the research on the effects of NO_3- during exercise have focused on endurance exercise with limited research on short-duration, maximal-intensity exercise performance (41). Given the importance of even slight performance improvements for athletes competing in high power sports, a safe, easily obtainable NO_3- product (such as beets/beet juice) could be a desirable element to include in one's pre-competition regimen. Anaerobically trained athletes differ in their performance measures relative to aerobically trained athletes. For example, sprinters are reported to reach power outputs that are three to five times greater than what is achieved at one's $\dot{V}O_2$ max (27). During a sprint-based shift in ice hockey, two-thirds of the energy used are supplied by anaerobic sources (9). In contrast, as much as 80% of the energy utilized during a four-minute bout of exhaustive exercise are supplied by aerobic energy sources (22). Therefore, the findings from studies focusing on primarily aerobic exercise bouts greater than two minutes in duration do not fully apply to shorter duration exercise that is primarily anaerobic in nature due to differences in power production and lower reliance on oxygen availability.

Evidence suggests that the NO_3- pathway is more active in hypoxic and acidic conditions, which are the conditions that occur during intense exercise (42). These findings have sparked an upswing in research that examines the potential ergogenic impact of beet juice supplementation on supramaximal work (2, 6, 14, 16, 17, 32, 38). Repeated sprint protocols often utilize short-duration, high-intensity intervals to examine overall performance across exercise sessions (2, 8, 40). However, fatigue plays a role with each subsequent sprint across the protocol duration and total work completed, reflecting a greater impact on aerobic capacity than anaerobic power (23).

Dietary NO_3-'s ability to produce performance benefits during short-duration, high-power activity may be mechanistically different from the benefits of NO_3- supplementation with aerobic exercise (40). The relationship and dual role that the aerobic and anaerobic energy pathways play during any bout of exercise is another important consideration. Gastin (22) suggested an approximately equivalent contribution of the aerobic and anaerobic energy pathways occurs at around 75 seconds of exercise. Very short-duration work (e.g. $\leq$ 30 seconds) utilizes a relatively low proportion of its ATP via aerobic energy pathways. Substantial evidence suggests that NO_3- supplementation improves the efficiency of oxidative metabolism (3, 4, 29, 42), thus even mostly anaerobic bouts of exercise could still benefit due to a small portion of aerobic pathway involvement. The purpose of this study was to determine if acute dietary NO_3- supplementation, in the form of beet juice, could improve measures of anaerobic power during a 30-s maximal-effort bout in anaerobically trained, adult men. With consideration that a longer exercise task would rely on a greater contribution of the aerobic energy pathways for ATP production and potentially a larger improvement in performance, a secondary purpose was to investigate if there was a greater increase in maximal intensity performance with beet juice supplementation during a 60-s maximal-effort bout compared to a 30-s bout. We hypothesized that measures of anaerobic power would not be significantly different during a 30-s maximal effort, but measures of anaerobic power would be significantly different during a 60-s maximal effort due to the greater contribution from aerobic energy pathways.

METHODS

Participants

Male hockey players between 18 and 45 years old with at least three seasons of competitive hockey experience and regular hockey-related training during the previous three months were recruited for this study. Hockey players were selected because of the higher reliance on anaerobic pathways during hockey training and gameplay (9). Participants were recruited from local club teams, USHL junior leagues, and adult A level competitive, recreational leagues. A priori power analysis indicated that for a mean difference of 85 ± 150W at a statistical power of 0.80, twelve participants were needed. A total of 14 subjects completed the study. Table 1 presents the characteristics of the 14 study participants. An Institutional Review Board-approved informed consent form and a Physical Activity Readiness Questionnaire (PAR-Q) (44) were completed by all participants prior to enrollment. Additional questions about hockey and exercise history were included to ensure that each participant was regularly engaging in high-intensity, primarily anaerobic exercise, as training status may influence the effectiveness of beet juice (37). This research was carried out fully in accordance to the ethical standards of the International Journal of Exercise Science (34).

Table 1. Participant baseline characteristics.

Age (years)	30.86 ± 7.55
Height (m)	1.82 ± 0.05
Weight (kg)	81.81 ± 7.95
BMI (kg/m^2)	24.78 ± 2.41
Body Fat (%)	14.58 ± 7.55
Hockey Experience (yrs)	16.64 ± 10.02

Note: Data presented as mean ± SD.

Protocol

This study utilized a randomized, double-blind, counterbalanced, placebo-controlled, crossover-study design. Each participant completed two familiarization trials and four experimental trials. Body mass was measured during each visit (Tanita, BWB-800, Tanita Corp., Tokyo, Japan) to determine the appropriate resistance for the Wingate tests.

Visit 1: First, body mass and body fat percent were measured using the BOD POD (COSMED, Concord, CA) (19). Each participant completed a 30-s Wingate familiarization trial during Visit 1. The protocol for each Wingate test began with a 10 to 15 minute warm up on a cycle ergometer at a self-selected intensity. During the last five minutes of the warm-up, the participants completed three 10-second (10-s) sprints to ensure the muscles were adequately prepared for the upcoming bout of high-intensity exercise. Following the preliminary warm up, the testing protocol consisted of “warm-up,” “ramp-up,” and “test” phases. The warm-up phase included pedaling for 20-s at approximately 100 RPM with a resistance of 100W. After 20-s, the participant was instructed to increase their pedaling cadence speed, ramping up to their maximal cadence (“ramp-up” phase) within 5-s. The test phase began immediately after the 5-s ramp-up phase. For the 30-s test, a resistance of 9.5% of the participant’s body weight was automatically applied to the flywheel at the onset of the test (21). The participant continued to pedal at a maximal cadence while remaining seated in the saddle throughout the test. At the end of the 30-s test, the resistance was automatically reduced to 100W, allowing the participant to cool down for a minimum of 10 minutes. Participants remained in the laboratory for at least 15 minutes following each test to ensure heart rate and blood pressure had recovered after the intense exercise bout.

Visit 2: Visits 1 and 2 were separated by at least 24 hours. Visit 2 consisted of a familiarization trial of the 60-s Wingate test. The 60-s Wingate protocol was similar to the 30-s test with only the duration and resistance applied being different from the 30-s test. The same 10-minute warm-up procedures were followed. As with the 30-s test, the 60-s test was broken into "warm-up," "ramp-up," and "test" phases. The warm-up and ramp-up matched the 30-s protocol, but the resistance applied at the onset of the 60-s test phase was 7.5% of the participant's body weight (21). The 60-s test was designed to maximally challenge the anaerobic pathways (i.e. ATP-CP and glycolysis) but would also require a greater reliance on the aerobic system than the 30-s test due to the longer duration (21).

Visits 3 through 6: After Visit 2, each participant was scheduled for the four experimental trials. During their familiarization sessions, participants were instructed on preparation for visits 3 through 6 including: maintaining a consistent diet in the 24 hour prior to a test day, keeping a 24-hour food record, education on specific NO3- containing foods to limit/avoid prior to a test day, avoiding commercial mouthwash and/or chewing gum on the day of a test (24), and avoiding alcohol in the 24 hours prior and caffeine in the 12 hours prior to a test. Subjects were asked to avoid consuming any food or drink for three hours prior to the test apart from water (ad libitum) and a standardized snack of a large apple (supplied by the researchers prior to the scheduled test day) that was consumed one hour prior to the test. Improvements in performance have been correlated to plasma nitrite (NO2-) levels. After beet juice consumption, plasma NO2- levels typically peak two-to-three hours post consumption and remain near peak levels until five hours post consumption (46). Therefore, consumption of the experimental beverage three hours prior to their scheduled test time ensured participants would be in the optimal window of time to experience any potential effects from beet juice supplementation. Participants were instructed to continue their normal exercise regimen and avoid any significant changes in training during their study involvement. They were asked to maintain the same exercise routine during the day prior to testing (verified by exercise log) and were instructed to refrain from exercise prior to each laboratory visit on the day of the test.

Visits 3 through 6 for each participant were randomized among four conditions: 30-s Wingate after consuming beet juice (B30), 30-s Wingate after consuming the placebo (P30), 60-s Wingate after consuming beet juice (B60), and 60-s Wingate after consuming the placebo (P60). A research assistant not involved in data collection managed the randomization process and prepared the appropriate supplement (beet juice or placebo) prior to each participant's trial. The supplement and the standardized snack were provided to the participant 24 to 72 hours preceding the trial. Supplements were prepared in standard containers and were placed in a brown paper sack to ensure double-blinding status.

Immediately prior to beginning each test, a saliva sample was obtained to evaluate NO level in the body. A NO saliva test strip (Berkeley Test, Chicago, IL) was placed on the tongue of the participant and held in the mouth for ~5-s, until sufficiently saturated with saliva. The strip was removed from the mouth, allowed to react for ~45-s, after which the strip's color would represent an estimated NO2- level in the saliva, which is considered representative of circulating NO levels (7). Test strips were assigned a value of 1 to 5+ based on the color rating provided by the manufacturer. Values were defined as follows: 1- depleted, 2- low, 3- threshold, 4- target, 5- high, 5+ (darker than the highest color on the range). A research assistant not involved during

Wingate testing administered the NO test strip assessments and recorded the results to maintain the double-blinded study design by those administering the Wingate tests.

Every attempt was made to schedule participants at or near the same time of day for each visit to control for diurnal rhythm effects on peak power (30). Participants confirmed compliance of consumption of the beverage three hours prior to their appointment by sending a time stamped photo after the supplement was consumed (11) and signing an attestation upon arrival to the lab for the test. The attestation also confirmed, to the best of their abilities, that they followed all other pre-trial instructions in the preceding 24 hours. A 24-hour diet and exercise recall was returned to a study investigator during Visits 3 through 6. During Visits 3 through 6, participants were randomly assigned to complete either the 30-s or 60-s Wingate test using the previously described familiarization trial protocol. Each of Visits 3 through 6 were separated by a washout period of at least 48 hours (47). All within-subject experimental trials occurred over a two- to ten-week period.

Beet Juice/Placebo Supplements: All of the beet juice supplements supplied by the manufacturer had the same lot number, ensuring that the beet powder was processed from the same batch of beets. One dose of red beet juice powder (RediBeets, The AIM Companies USA, Nampa, ID) containing ~8 mmol (496 mg) NO3- (Analytical Laboratories, Boise, ID) mixed with 237 mL of water was utilized for dietary NO3- supplementation. This dose is considered "high" (high > 7.5 mmol) (35) and was higher than NO3- doses used in previous studies that found performance benefits during endurance cycling exercise (28, 43). An equivalent-volume cherry-apple-cranberry juice blend prepared by a registered dietitian was used for the placebo trials. The combination of cherry, apple, and cranberry juice creates a product similar in color to beet juice, supporting the double-blinded nature of the study. The placebo juice contained a trivial amount of NO3- per dose (~0.98 mg) (Analytical Laboratories, Boise, ID). The placebo and beet juice supplements were isocaloric and contained similar amounts of carbohydrate (Table 2). Cherry-apple-cranberry juice and beet juice do not have identical nutrient profiles, but most fruits are low in NO3- content and thus appropriate for use as a placebo (33). In all study documentation provided to participants, they were informed that the study was "assessing the exercise performance benefits of different natural, nutritional beverages." Details about the experimental design or the nutritional contents of either the experimental or the placebo beverages were not provided to the participants until study-related data collection were completed.

Table 2. Nutrient content of beet juice and placebo juice.

Nutrient	Beet Juice	Placebo
Kcal	105	108
Carbohydrate (g)	21	25
Protein (g)	7	0
Fat (g)	0	0
Sodium (mg)	105	41
NO_3- (mg)	496	0.97

Short-duration Power Assessments: The 30-s and 60-s Wingate cycle ergometer tests were utilized to evaluate short-duration, maximal-intensity power. The 30-s Wingate is a maximal-exercise test that is reproducible and considered to be a valid method of assessing power output during primarily anaerobic exercise (25). A 60-s variation of the Wingate was also completed by each subject (21). The exercise trials were completed on the Velotron Pro, using a Dynafit bike frame (RacerMate Inc., Seattle, WA) with an 86-tooth chain ring that was adjusted ensuring that saddle height/fore-aft position and handlebar height/fore-aft position were comfortable for the participant. Velotron Wingate Software, Version 1.0 (Racer Mate, 2010) was utilized for data collection during the tests. Data variables obtained during the Wingate protocol included minimum, peak, and mean measurements of: absolute power (W), relative power (W/kg), and RPM. For each variable, "peak" was defined as the highest value achieved over the course of the test (generally occurred during the first few seconds of the test) and "mean" was the average value over the course of the 30-s or 60-s test. Fatigue index (FI) (%) was also calculated for each test. FI was determined by calculating the difference between the peak power and the minimum power divided by the peak power. This value represents the reduction in power output from the point of the peak power output to the point of the lowest power output. A lower FI reflects a greater ability to maintain power output over the course of the test (i.e. lower rate of fatigue).

Statistical Analysis
All data were reported as mean ± standard deviation (SD). Analysis was completed using paired t-tests for variables from the B30 and P30 trials and the B60 and P60 trials to assess if beet juice consumption affected the performance measures. In addition, Repeated Measures (RM) ANOVA was completed for power output using the 10Hz data for each trial. Percent change between the beet and placebo trial was calculated for each variable of the 30-s and 60-s tests. A one-way ANOVA was run on the percent-change values to determine if greater effects occurred in the 60-s test compared to the 30-s test. Effect sizes (ES) were determined for each variable measured in the 30-s and 60-s tests. ES was calculated using the following formula: (Beet Mean – Placebo Mean) / Pooled Standard Deviation. ES of 0.2, 0.5, and 0.8 were considered small, medium, and large, respectively with ES of 0.2 or less considered to be trivial (15). All analyses were completed using SPSS, Version 24 (IBM, Armonk, NY), with statistical significance set as an alpha level of 0.05.

RESULTS

Seventeen participants volunteered for this study. Oversampling beyond the a priori calculated sample size was completed to account for the possible attrition of subjects over the course of the study. Three participants completed only one of the study trials and were not included in the final data analysis. With the exception of the 10Hz data, all data analyses were completed on the 14 participants who completed all six study visits (Table 1). Most participants tolerated the consumption of the NO_3^- supplements well. However, one participant complained of mild-to-moderate abdominal cramping on two occasions and another participant experienced mild nausea and diarrhea on one occasion after consuming the beet juice. RM ANOVA analysis of the 24-hour food recalls revealed no significant differences in caloric intake between the 30-s beet juice/placebo trials (p = 0.335) or the 60-s beet/placebo trials (p = 0.234). A one-way

ANOVA revealed no significant changes in body weight for the participants across the study period ($p = 0.123$).

NO2- Level: All participants confirmed consumption of the supplement three hours prior to their scheduled test by sending a time stamped photo of the empty supplement container (11). In addition, participants signed a confirmation of adherence to all pre-testing guidelines during each study visit. NO2- salivary test results provided confirmation that NO2- levels increased as expected after consumption of the beet juice and remained low after consumption of the placebo. Using the color-rating scale provided by the test-strip manufacturer (1 = "depleted" and 5 = "high"), the mean NO2- test values were 1.36 ± 0.48 and 1.46 ± 0.45 for the P30 and P60 tests respectively. The NO2- values prior to the B30 and B60 tests indicated mean values of 4.11 ± 1.09 and 4.50 ± 0.91, respectively. Paired t-tests revealed significant differences between NO2- levels in the placebo and beet juice conditions for both the 30-s and 60-s tests ($p < 0.001$).

Short-duration Power Performance: An order-effect analysis was completed to determine if participants improved in performance measures as they progressed through the testing phase regardless of the randomization protocol. A comparison of performance between the familiarization trials, and each subsequent visit for each the 30-s and 60-s test revealed no significant differences ($p > 0.05$), indicating that an order effect was not present for the group of tests.

30-s Wingate Performance: Analysis of the B30 vs. P30 data indicated no statistical differences across any of the variables measured. Maximal power was reached within the first second of the test and declined over the course of the test with the minimal power produced in the final seconds. A trend emerged for a lower FI (associated with a decreased rate of fatigue over the test) after beet juice consumption relative to placebo but it did not reach statistical significance ($p = 0.059$). Figure 1 presents the data from the 30-s Wingate test for comparison of power (Figure 1A; peak, mean, and minimum), pedaling speed (Figure 1B; peak, mean, and minimum), relative power (Figure 1C; peak and mean) and FI (Figure 1D) for each of the 14 study participants. Data were analyzed using the raw 10Hz data for the entire 30-s test. Due to lost data files from the hard drive, only the complete data sets for eight of the subjects were included in the 10Hz data analysis. RM ANOVA indicated no overall difference between the beet juice and placebo trials ($p = 0.380$) (Figure 2). When considering ES, the beet juice had the greatest performance impact on minimum power (W) and minimum RPM (ES = 0.26 and 0.22). Beet supplementation was also shown to yield a lower FI (ES = -0.31). All other ES values were trivial (ES < 0.20).

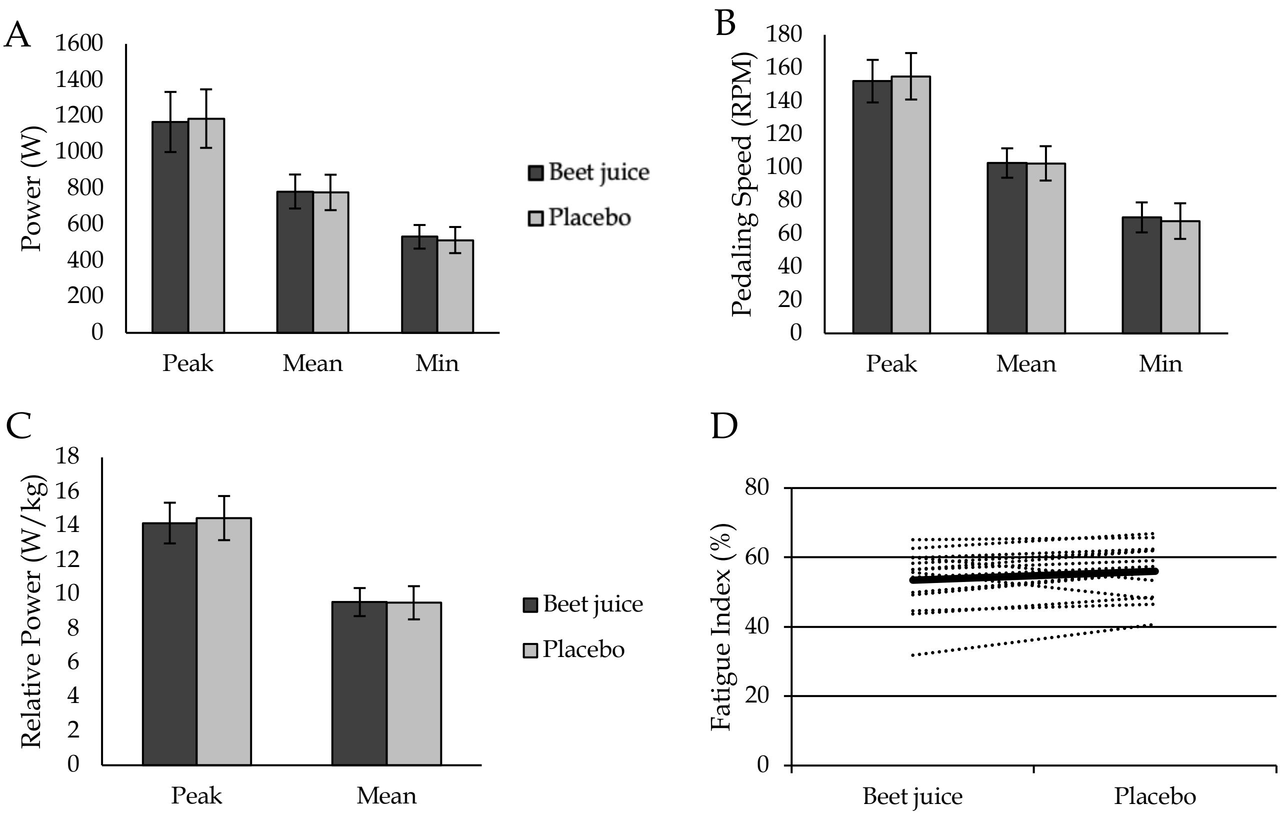

Figure 1. Changes in power (A), pedaling speed (B), relative power (C) and Fatigue Index (D) during 30-s trial after consumption of beet juice vs. placebo (n = 14). In Panels A-C, data represent group mean ± SD. In Panel D, a higher FI % indicates a greater rate of fatigue that occurred over the course of the test. Dotted lines represent individual participant responses. Mean values are represented by the solid line. There were no significant differences between beet and placebo conditions ($p > 0.05$).

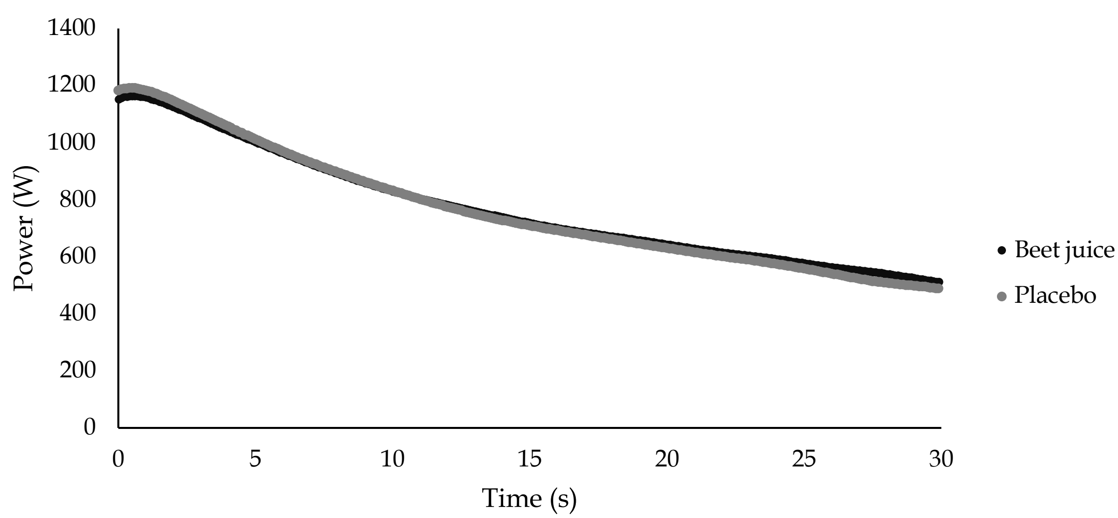

Figure 2. Changes in power after consumption of beet juice vs. placebo during the 30-s test from the 10Hz data (n = 8). There was no overall significant difference between the beet juice and placebo conditions (p = 0.380).

60-s Wingate Performance: Analysis of the B60 vs. P60 data indicated no statistical differences across any of the variables measured. Figure 3 presents the data from the 60-s Wingate test for comparison of power (Figure 3A; peak, mean, and minimum), pedaling speed (Figure 3B; peak, mean, and minimum), relative power (Figure 3C; peak and mean) and FI (Figure 3D) for each of the 14 study participants. As previously mentioned, the analysis of the 10Hz data for the 60-s test was completed on eight of the original 14 participants. RM ANOVA indicated no overall difference between the beet juice and placebo trials (p = 0.093) (Figure 4). As with the 30-s test, during the 60-s test maximal power was reached within the first second and decreased over the duration of the test with minimal power produced in the final seconds (Figure 4). The ES was trivial (< 0.20) for all variables when considering beet juice supplementation's effect on performance variables during the 60-s test.

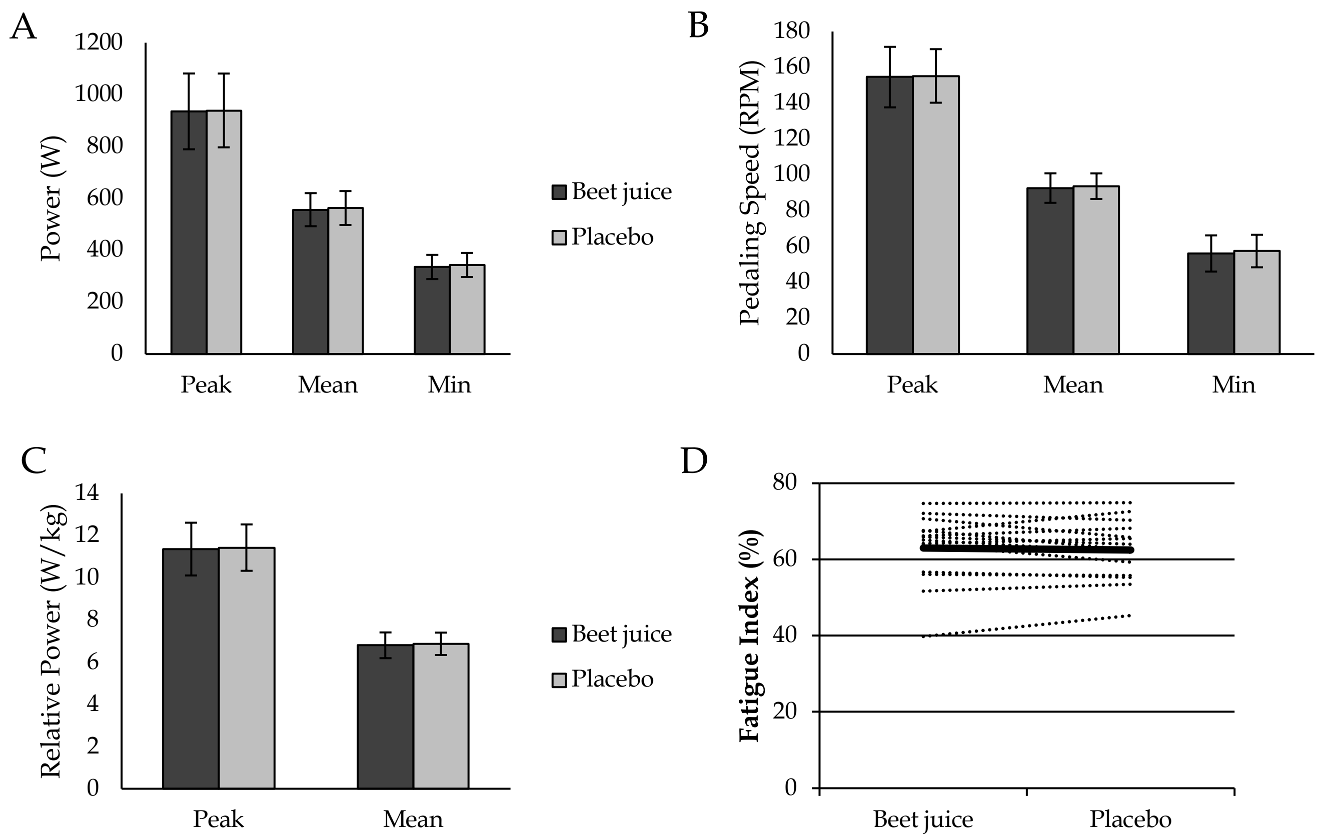

Figure 3. Changes in power (A), pedaling speed (B), relative power (C) and Fatigue Index (D) during 60-s trial after consumption of beet juice vs. placebo (n = 14). In Panels A-C, data represent group mean ± SD. In Panel D, a higher FI % indicates a greater rate of fatigue that occurred over the course of the test. Dotted lines represent individual participant responses. Mean values are represented by the solid line. There were no significant differences between beet and placebo conditions (p > 0.05).

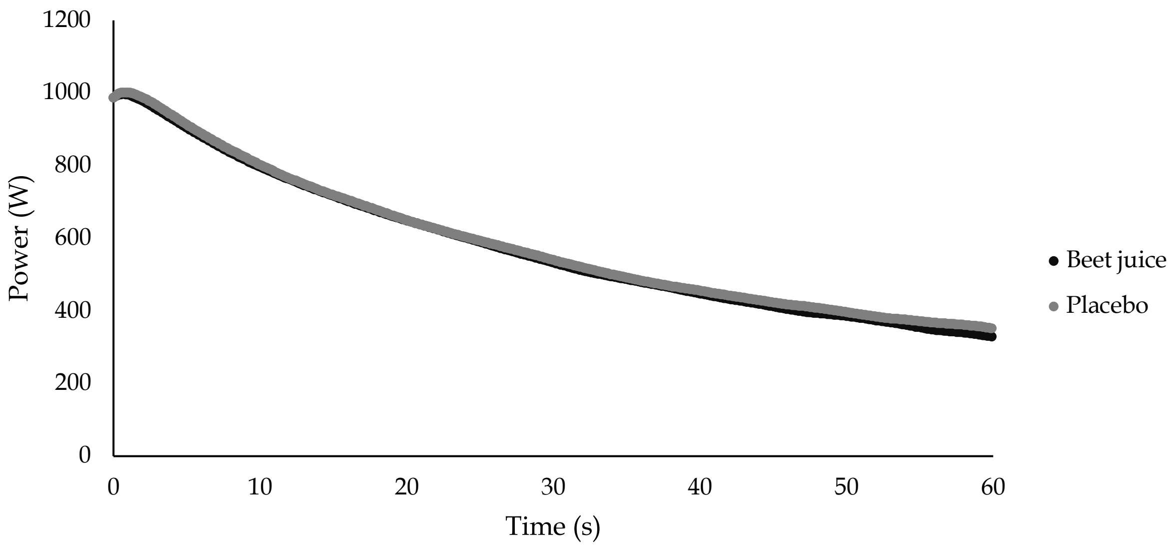

Figure 4. Changes in power after consumption of beet juice vs. placebo during the 60-s test from the 10Hz data (n = 8). There was no overall significant difference between the beet juice and placebo conditions (p = 0.093).

Comparison of 30-s vs 60-s Tests: To compare the performance changes between the 30-s and 60-s tests, the percent change (Δ) between the experimental and placebo supplements was calculated for the 30-s and 60-s test. The FI Δ was significantly different when comparing the 30-s and 60-s tests (p = 0.032). The FI decreased between the B30 and P30 (53.44 ± 8.88% vs. 56.01 ± 7.79%) while it increased between the B60 and P60 (62.49 ± 8.11% vs. 63.01 ± 9.19%). No other significant differences emerged when comparing the percent change between the 30-s and 60-s tests. Key measurements are presented in Table 3.

Table 3. Comparison of the percent change in the beet and placebo trial between the 30-s and 60-s tests.

	B30 vs. P30 Δ (%)	B60 vs. P60 Δ (%)	p-value
Mean Power (W)	0.69 ± 4.34	-1.09 ± 4.46	0.396
Mean RPM (RPM)	0.32 ± 4.57	-1.24 ± 4.89	0.485
Relative Mean Power (W/kg)	0.55 ± 4.62	-1.20 ± 4.75	0.429
Relative Peak Power (W/kg)	-1.96 ± 7.37	-0.69 ± 6.33	0.634
FI (%)	-5.24 ± 9.70	0.50 ± 5.94	0.032*

Data presented as group mean ± SD. For all variables with the exception of FI, a positive **Δ** indicates that the beet juice improved performance, a negative **Δ** indicates that the placebo improved performance. For FI, a negative **Δ** indicates a greater capacity to maintain power throughout the test during the beet juice trial. *Statistically significant (p < 0.05).

DISCUSSION

The results of this study suggested that beet juice supplementation did not improve performance during a 30-s or 60-s maximal intensity test on a cycle ergometer. Additionally, beet juice supplementation did not significantly influence performance during the 60-s test to any greater extent than its impact in the 30-s test. The difference in the Δ between the beet vs.

placebo FI in the 30-s test when compared to the beet vs. placebo FI in the 60-s test did reach statistical significance ($p = 0.032$). This finding suggests that less fatigue occurred (i.e. improved ability to maintain power) in participants who consumed beet juice compared to the placebo in the 30-s test, when compared to the fatigue that occurred in participants who consumed beet juice compared to the placebo in the 60-s test. While this would suggest that there is an ergogenic benefit to consuming beet juice during a 30-s sprint, it was not well supported by the other findings in this study and it may have limited clinical relevance. However, it is an area that should be further investigated in future research.

Previously, only two studies have evaluated the effect of a single dose of beet juice on maximal intensity performance during a 30-s Wingate test (17, 38). Using a population of trained athletes from a variety of sports, Rimer et al. (38) had participants complete a series of four maximal cycling efforts (3-4 s) with two minutes of recovery between each trial. Then, participants rested for five minutes before completing a 30-s Wingate test (38). The authors found an improvement with beet juice consumption (~11.2 mmol NO3-) in peak power output during the 3-4 s sprints, but no significant differences in peak power output, total work completed, or rate of fatigue of the 30-s Wingate test (38). While the Rimer et al. (38) study was similar to the present study in that they utilized a single dose of beet juice, it differed significantly in the study methodology. Rimer et al. (38) used a repeated sprint protocol, which does not allow for complete recovery prior to the subsequent sprints. Therefore, there is limited relevance to the present study. Using a protocol that was more similar to that used in the present study, Dominguez et al. (17) asked participants to complete a single 30-s Wingate test after consuming beet juice or a placebo. Despite utilizing a NO3- dose that was lower than the dose used in the present study (~5.6 mmol NO3- vs. ~8 mmol NO3-), the authors found that beet juice consumption significantly improved peak power output and power output during the initial 5-s of the trial compared to placebo (17). While Dominguez et al. (17) reported that the participants in the study were "trained," upon closer inspection it was clear that the training status of the sample population was lower than the present study with overall peak power outputs of 841 W compared to 1,177 W in the present study. The lower resistance used by Dominguez et al. (17) (7.5% vs. 9.5% of body weight) could partially explain the peak power output differences during the 30-s Wingate. However, the 60-s Wingate protocol in the present study used the same 7.5% of body-weight resistance and the participants also generated higher overall peak power (937 W) that the 841 W reported by Dominguez et al. (17). Previous research has found that well-trained athletes experience less of a rise in plasma NO2-, and less of a performance benefit (45). Thus, the present study is consistent with previous reports that suggest that beet juice may be less effective in well-trained athletes (26, 36, 38, 45).

Although investigating the mechanistic theories explaining the benefits of dietary NO3- on exercise performance were not the focus of this study, a consideration of these theories provides important insight into the lack of significant improvements found in the present study. It has been suggested that beet juice consumption before exercise may have the ability to (1) reduce the O2 cost of ATP resynthesis, thus increasing the mitochondrial phosphate/oxygen ratio, (2) reduce the ATP cost of force production and reduce the degradation of PCr during exercise, and/or (3) inhibit ATP production with a resulting increase in energy provided via anaerobic pathways (3, 4, 29). If the first two theories were correct, a single bout of high-intensity exercise (e.g. a 30-s or 60-s Wingate test) would likely not benefit from these physiologic responses. However, NO3- supplementation before repeated bouts of high-intensity exercise could be beneficial due to the greater reliance on the aerobic-energy pathways and phosphocreatine preservation (31). The third theory is also not well supported for a single, primarily anaerobic bout, as several studies have found no differences in lactate production between the NO3- and placebo supplemented groups (2, 29, 46). Thus, one would not expect to observe improvements in short-duration, maximal-intensity performance with the consumption of beet juice.

Thompson et al. (39, 40) utilized intermittent exercise protocols to investigate beet juice's role on sprint performance. When repeated 6-s sprints on a cycle ergometer were analyzed, beet juice significantly improved individual sprint time in five of the first 20 sprints (2nd, 5th, 7th, 8th, and 13th sprint) when compared to the placebo (40). In a follow up study, performance during five 20-m running sprints was examined with beet juice improving run time by 1.2% compared to the placebo (39). However, one could argue that based on the inconsistent results across all sprints (40) and the minor improvement in run time performance (39), these results suggest that the improvements in sprint performance with NO3- supplementation were inconsistent. While Thompson et al.'s (39, 40) studies revealed inconsistent results in sprint performance following five to seven days of supplementation, no improvements in peak power were observed after a single dose of beet juice in the present study.

There are several strengths of the present study. The crossover design helped to ensure limited subject variability when comparing the experimental to the placebo conditions. Maximal-intensity performance was assessed using the Wingate test on a magnetically braked cycle ergometer, which is considered an effective tool to measure short duration, maximal intensity performance (10, 18). Multiple familiarization trials assured a learning effect would have a limited, if any, impact on the data (5). Diet, exercise, and diurnal rhythm influences were well controlled. A double-blinded design was maintained throughout the study duration. The NO3- supplementation was a palatable dose, in the form of beet juice that could realistically be consumed by athletes prior to an event. The placebo provided a negligible NO3- amount, and the expected increase in circulating NO2- after consuming the beet juice, with a lack of an increase after consuming the placebo juice, occurred and was confirmed via saliva NO2- measurements.

However, this study did have some inherent limitations. With only 14 participants completing all trials, the sample size could be considered small. However, this sample size is comparable to other beet juice performance studies (2, 8, 17, 28, 38-40, 46). A priori power analysis indicated that a sample size of 12 participants would have been needed to achieve a statistical power of 0.80. Because of the loss of some of the data files, the sample size for the analysis of the 10Hz data in both the 30-s and 60-s tests were under powered (n = 8). Therefore, the lack of significant differences reported from the analysis of the 10Hz data should be interpreted with caution. Although salivary NO2- values were assessed, another limitation is the lack of plasma NO3- values. Peak muscular torque is positively correlated with plasma NO3- values (13). Although salivary NO2- values are positively correlated with plasma NO3- values (r = 0.634) (12), it is possible that the plasma NO3- values were inadequate to influence increases in anaerobic power. As with any performance study, day-to-day variations in performance could have played a role in the present study. However, the intra-individual consistency of the performance results in the present study suggest that day-to-day variation was minimal. With these limitations noted, this study adds novel information to the existing body of research on beet juice as an ergogenic aid.

The dose of the NO3- source (RediBeets) utilized in this study was greater than the recommended serving size by the manufacturer. The manufacturer reported that a one tablespoon (4 g) serving size contained around 72 mg of NO3- (1.16 mmol) (1). In order to achieve our goal of ~8 mmol, our dose contained 28 g of RediBeets. While this dose was around seven times greater than the manufacturer's suggested serving size, the manufacturer suggests a minimum of 300 mg for "enhanced athletic performance" (1). Recently, Gallardo and Coggan (20) reported on the NO3- content of beet juice products. The authors' reported that the measured NO3- in RediBeets was 0.43 mmol/serving (i.e. ~3.01 mmol/dose utilized in the present study) (20). This would suggest that the actual amount of NO3- in the dose utilized in the present study was less than half of our reported concentration. Acknowledging that there could be variations in NO3- levels from one batch to the next, a sample from the powdered beet juice supplement batch utilized for all NO3- supplements in the present study was independently analyzed and found to be within the target NO3- range of ~8 mmol (496 mg) (Analytical Laboratories, Boise, ID). Nevertheless, the results reported by Gallardo and Coggan (20) are important to consider. Based on their results and our independent analysis of samples, there appears to be considerable variation in the NO3- levels across samples from this manufacturer. However, the NO3- dose utilized in the present study was within the suggested range for ergogenic benefits (35).

In conclusion, a dose of ~8 mmol of beet juice did not improve short-duration, maximal-intensity exercise performance during a 30-s or 60-s Wingate test. No significant differences were found in most of the performance variables when comparing the placebo and beet juice trials. In addition, performance during a longer bout of anaerobic work (60-s vs. 30-s) was not impacted to a greater extent than a shorter bout after beet juice supplementation. This suggests that the benefits of beet juice supplementation observed during aerobic work are not apparent during a maximal intensity bout of exercise that requires less than 50% of energy from the aerobic pathway (22).

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