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The Effect of Beetroot Supplementation on High-Intensity Functional Training Performance

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ABSTRACT

International Journal of Exercise Science 13(2): 667-676, 2020. Nitrate supplementation (NO_3^-) has been shown to improve athletic performance for short-duration, vigorous activity, as well as long-duration, aerobic activity. The purpose was to explore the effects of beetroot supplementation (BR) on high-intensity functional training (HIFT) performance. Twenty-four HIFT participants (25 ± 6.5 years, 175.17 ± 8.1 cm, 84.94 ± 12.09 kg), who attended HIFT classes at least 3 days per week for the past 3 months, performed a benchmark performance test (5 rounds of a 400-m run followed by 15 overhead squats with a 95-lb (for males)/65-lb (for females) barbell). In a randomized order, 72 hrs apart, participants were tested under a control session and once after consuming 70 mL beetroot nitrate supplement, Beet It®, 2 hours prior to beginning the assigned benchmark test. For both benchmark tests, time to completion, pre- and post-exercise blood lactate levels, RPE, and pre-, during, and post-exercising heart rates were measured. There was no significant difference ($p < 0.05$) between the control (930 ± 192.6 sec) and supplement (952.8 ± 205.8 sec) on time to complete the performance test. Post-exercise blood lactate (11.14 ± 2.84 mm/dL) was not significantly different ($p < 0.05$) than the control (12.00 ± 2.53 mm/dL). Additionally, mean RPE for BR supplement (14.78 ± 2.50) was not significantly different ($p < 0.05$) than the control (14.92 ± 2.12). The short duration and high intensity of the workout, which included both anaerobic and aerobic components, may have mitigated the cardiovascular effect of beetroot nitrates unlike previous research that found significant positive effects between beetroot nitrates and exercise performance.

KEY WORDS: Dietary nitrates, HIIT, nitrate supplementation

INTRODUCTION

Antioxidants and their role during exercise have been studied thoroughly because of the oxidative stress that occurs during and after an exercise bout (2, 3, 8, 9, 11, 12). During exercise, free-radicals are produced as a byproduct of metabolisms used to create energy (11). An accumulation of free-radicals can increase cellular damage by disrupting enzymatic signaling within the metabolic processes. One of the roles of antioxidants is to buffer free-radicals by binding to them and clearing them until the high demand for metabolic reactions is decreased (9, 13, 23). Antioxidants, such as nitrates, reduce the ability of free-radicals to affect the cell, and as a result help to improve exercise performance by promoting energy metabolism (9).

Nitrates may also improve performance by improving both blood flow and antioxidant buffering of free-radicals (13). By enhancing blood flow, the rate muscles receive oxygen is increased and athletic performance and recovery can improve. When muscles receive more oxygen, they are able to continue aerobic metabolic pathways. This allows the waste products of those processes to be reused for additional energy unlike during anaerobic metabolism where those waste products accumulate and cause muscle fatigue (5, 22). Additionally, BR- induced increases in [NO] may reduce excessive Ca^{2+} release from the sarcoplasmic reticulum and reduce the energy cost of subsequent resequestration (2). As such, nitrates from a diet rich in vegetables has been shown to improve the oxygen cost during submaximal exercise by 3-6% (6, 13, 23). Nitrates help increase blood flow, muscular contractions force, and glucose uptake during exercise which can improve performance (3, 6, 24, 25).

Dietary NO_3^- is reduced in the mouth via the microbiome production of NO_3^- reductase to nitrite (NO_2^-). NO_2^- is continued to be reduced through the acidic environment of the stomach into NO where it is then absorbed in the gut increasing the plasma [NO]. Some NO_2^- is absorbed in the mouth as well as in the gut, which, under low oxygen conditions, reduces further to NO (5, 6). Once in the plasma, through soluble guanylyl cyclase mediated reaction, NO stimulate smooth muscle fibers of the blood vessels to dilate (20). Through vasodilation, there is an increase in blood flow and enhanced gas exchange to skeletal muscle (5, 6).

Supplement concentrates offer the benefits of beetroot nitrates (NO_3^-) in an easier and more convenient consumable form. Beetroot nitrates supplementation improves performance during moderate, long-duration events by significantly reducing oxygen cost (3, 19). Oxygen uptake improves following beetroot nitrate supplementation (BR), which is believed to be from increased blood nitrous oxide (NO) concentration. The BR-induced increase in [NO] caused vasodilation as well as increased mitochondrial oxidative phosphorylation which prevented "slippage" of the proton pumps (2, 3). Additionally, vasodilation caused the blood vessels around the muscle to increase in size, which allowed more blood to flow to the muscle, increasing oxygen supply to continue producing repeated contractions. The increased blood-[NO] has also been linked to aerobic respiration efficiency, regulated muscle contractions, and increased time to exhaustion (24).

Researchers have also found that BR improves intense, short-duration exercise (2, 8, 24). Significant improvements in time-to-exhaustion were seen as well as a reduction in ATP and phosphocreatine cost of muscle force production (2, 8, 24). ATP, phosphocreatine, and anaerobic glycolysis are the main sources of energy during short, intense exercise bouts, therefore, by reducing their involvement to produce the same muscular force can extend the time until task failure. Blood flow improvements were also seen to influence the type II fibers, which produce the fastest and largest muscular forces recruited during intense exercise (24), thus improving efficiency.

High-intensity interval training (HIIT) consists of short bursts of vigorous activity with brief periods of rest or low-intensity aerobic exercise (7). This intermittent nature of activity and brief rest periods are thought to be the reason for the benefits in performance following beetroot supplementation. High-intensity functional training (HIFT), however, stresses functional, multi-joint movements at high intensities that may or may not incorporate rest periods (7). HIFT typically involves a variety of modalities, such as aerobic running combined with weight training within the same workout designed to complete as many rounds as possible or in the fastest time possible. At this point there are no studies looking at HIFT performance with BR supplementation nor, to our knowledge, the effect of BR supplementation on performance between males and females. The purpose of this study is to determine the acute effects of beetroot supplementation on HIFT performance. It is hypothesized that there will be an improvement in time to completion and/or decreased blood lactate levels after exercise from the dietary nitrate supplementation.

METHODS

This study was a between subjects repeated measures design to examine the effect of BR supplementation on the performance of a HIFT benchmark test. Subjects performed two counterbalanced trials of 5 rounds (sets) of a 400-m run followed by 15 overhead barbell squats (Rogue, Columbus, Ohio) with 43.09 kg for males and 29.48 kg for females, once with a control and another after consuming 70 mL (~4.2 mmol NO_3^-) of beetroot concentrate nitrate supplement 2 hours prior (23). The time differences between both trials were compared, as well as heart rate (HR), lactate levels, and subject's RPE.

Participants

The participants of this study passed the physical activity readiness questionnaire (PARQ+) to confirm there was no history of diseases that may impair exercise performance or tolerance of high intensity exercise. Subjects also completed a demographic questionnaire confirming that they were between the ages of 18-45 years old and if female, were not pregnant. Subject's also confirmed that their current physical activity level to meet the study qualifications was to have attended HIFT classes at least 3 times a week for at least the last three months.

There were 24 participants in this study (15 males: 24.8 ± 5.23 yrs, 179.3 ± 6.55 cm, 90.8 ± 10.6 kg, 12.28 ± 3.48%BF; 9 females: 26.22 ± 9.35 yrs, 168.3 ± 5.37 cm, 75.17 ± 7.19 kg, 28.49 ± 3.49% BF) with no past injury history including lower-limb injury and back pain or injury. Subjects were not permitted to perform exhaustive physical activity at least 24 hours prior to participating, they consumed no caffeine 2 hours prior to participating as well as no additional ergogenic pre-workout supplements. The study was approved by the Institutional Review Board of the University of Lynchburg. Additionally, this research was carried out fully in accordance to the ethical standards of the International Journal of Exercise Science (18)

Protocol

Subjects attended three sessions. During the first session, subjects read and signed the approved informed consent, filled out the PARQ+, and a demographic questionnaire confirming their eligibility to participate. Subject's height (Stadiometer; Seca, Hanover, Maryland) and weight (Scale; Tanita, Arlington Heights, Illinois) were measured as well as body composition using the skinfold caliper technique (Lange Skinfold Caliper, Cambridge, Maryland). The three-site method was used for skinfold measurements, females were measured on the triceps, suprailiac, and thigh, while males were measured on the chest, abdomen, and thigh. The three locations were measured and marked first, then each skinfold was pinched and measured three times in rotating order. To find the percent of body fat (%BF), the measurements taken from the skinfold measurements were inserted into the three-site Siri equations to produce body density and percent body fat for each individual (1).

After the first session, subjects received either a 70 mL control (strongly flavored water, Mio, Kraft Foods, Chicago, IL) or the beetroot nitrate supplement (Beet It Sport, Ipswich, UK), counterbalanced and single-blinded. The subjects were instructed to consume the beverage 2 hours prior to the scheduled exercise session (24). On the next session, subjects performed a HIFT workout consisting of 5 sets of running 400 meters performing 15 overhead squats (43.09 kg for males, 29.48 kg for females, respectively) on a continuous running clock. After the completion of the first session, subjects scheduled their final session with at least 72 hours between the two sessions and they were given the opposite supplement and were again instructed to take the supplement 2 hours prior to their next session.

Participant descriptive demographic data that was collected before participation in the study included; height, weight and body composition, using skinfold calipers. Lactate levels measured via finger capillary draw (Nova Biomedical, Chesire, UK) before performing and immediately following the exercise test. Heart rate (Polar, Lake Success, New York) was measured before and immediately after each round, while RPE was measured using the Borg scale of 6-20 after completion of the exercise. Measurements will be taken during both the control and the beetroot nitrate supplement trials.

Statistical Analysis

Three 2x2 between-subjects repeated measures analysis of variance were used to analyze the pre-post performance differences between the supplement trials and sexes for time to completion (sec), lactate (mmol/), and RPE using IBM SPSS 25 (IBM, Armonk, NY) with JASP 9.2 (Amsterdam, Netherlands). A 2X6 between subjects repeated measures analysis of variance was used to analyze the baseline and 5 round HR between the supplement trails and sexes. A Bonferroni post-hoc analysis was utilized for significant findings. For all analyses, the alpha level was set to 0.05 *a priori*. A power analysis, using G*Power (v3.1, Universitat Kiel, Germany) determined that 22 participants were needed in the present study for a power of 0.80, with an effect size of 0.5 and an $p = 0.05$.

RESULTS

There was no significant interaction ($F(1, 21) = 1.74$, $p > 0.05$) in time to complete the exercise test between the trials and sexes (BR: male 15.24 ± 2.01min vs female 16.90 ± 4.88min; Con: male 14.71 ± 1.79min vs female 16.66 ± 4.68min; Figure 1). Similarly, there was no significant interaction ($F(18,1) = 0.70$, $p > 0.05$) between pre and post lactate levels between sexes. However, there was between subject significant difference ($F(18,1) = 8.82$; $p < 0.05$) between the lactate levels of the sexes (Figure 2). Additionally, there was a significant main effects difference ($t(1) = -14.38$, $p < 0.05$) between pre- and post- exercise lactate levels (Figure 2).

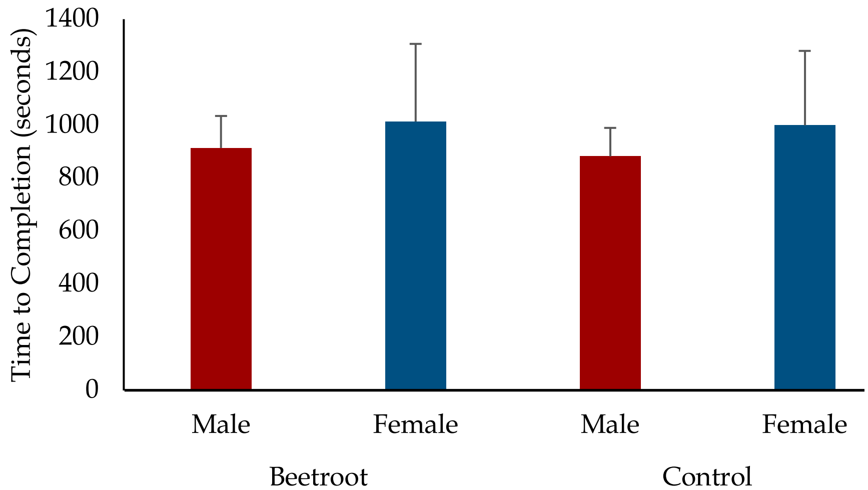

Figure 1. Time to completion of the exercise test.

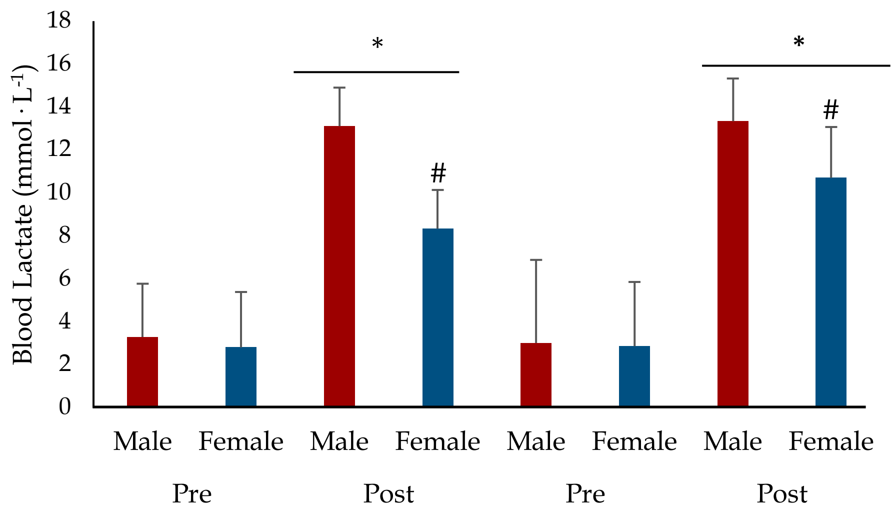

Figure 2. Blood lactate levels (mmol · L^{-1}). * Significant ($p < 0.05$) difference in lactate pre- and post- test. # Significant ($p < 0.05$) difference between sexes post-exercise lactate levels.

There was no significant interaction ($F(21,1)= 4.17$; $p > 0.05$) between the supplement and sex post- trial RPE. Additionally, there was no significant interaction ($F(55,5) = 0.66$, $p > 0.05$) between supplement, time and sex for HR over the trial. However, there was a significant ($F(55,5) = 231.11$, $p < 0.05$) difference in HR over time (Figure 3).

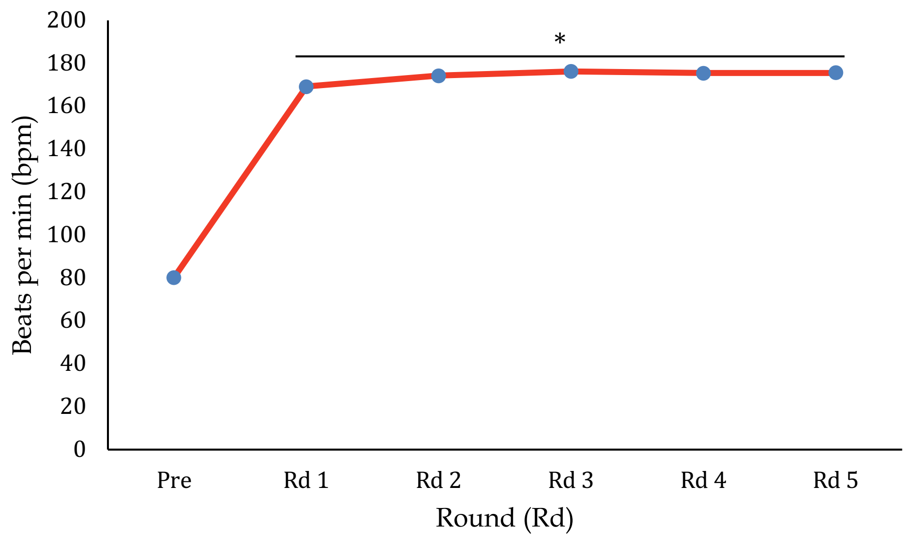

Figure 3. * Significant ($p < 0.05$) difference between pre- HR and post round HR.

DISCUSSION

During high-intensity exercise, fatigue is typically understood to be caused by insufficient energy supply to the working tissue in connection with the accumulation of intramuscular metabolic by-products (Ca^{2+}, H^+, and P_i) (14). Under aerobic and anaerobic conditions, the ability to offset these issues through increased blood flow has been shown to improve performance (10, 15, 22). Systemic nitric oxide (NO) levels play an integral role in regulating blood flow and mitochondrial respiration through vasodilation of the smooth muscles in the arterioles and improved oxygen uptake kinetics in favor of aerobic bioenergetics, thus mitigating the onset of fatigue caused by anaerobic metabolism (25). Additionally, NO also augments muscle contractility and calcium homeostasis through superior Ca^{2+} release and reuptake from the sarcoplasmic reticulum allowing for maintained muscular force production (25). As such, dietary nitrate (NO_3^-) consumption increases the plasma concentration of nitrites (NO_2^-), which, through future reduction, leads to an increase in plasma [NO] following absorption through the gut resulting in an increase oxygen availability to the tissue through vasodilation and sustained muscular force production (24).

The current study, however, found no significant differences between any of the measured dependent variables showing no effect between beetroot nitrate supplementation and HIFT performance. Unlike past studies (2, 3, 8, 11, 12) where beetroot nitrate supplementation had an effect on the time-to-exhaustion and oxygen cost of exercise this research examined a different version of high-intensity exercise, HIFT. The workout included both aerobic and anaerobic demands which may have counteracted each other and caused the BR to either not have or not show an effect on the performance, possibly due to the anaerobic load as seen by the rise in blood lactate concentrations. During the anaerobic period of the workout, the overhead squats, lactate would accumulate at an accelerated rate in the working muscles due to increased demand for ATP and reduced oxidative metabolism to assist with the clearing and reusing of lactate. However, between each set of overhead squats, the 400-m run would be an aerobic activity where the lactate that had been accumulated would be cleared and used to continue metabolic processes while the reperfusion of the capillary beds and vasodilation of the arteriole increase the clearance and support oxidative metabolism.

Previous studies (3, 11) that have looked at beetroot nitrates and the effect on oxygen cost found that long duration, lower intensity exercise benefitted from supplementation by reducing oxygen cost by ~3-6% (6). Similarly, when HIIT exercise modalities were examined, beetroot supplementation showed positive improvements in power and sprint time (4, 15, 16, 25). These intermittent exercise modalities, however, incorporated some designated rest periods between efforts, whereas this study had no designated rest periods. It may be that these periods of reduced muscular contractions allow for increases in oxygen availability, through increased blood flow to the tissue, that HIFT did not allow for.

The HIFT workout consisted of five 400m runs followed by weighted overhead squats. While the workout took around 15 minutes to complete, demonstrating potential aerobic properties, the high level of lactate and HR response indicated a much higher anaerobic demand than the HIIT studies (4, 15, 16, 25). Mosher et al (15) reported post-exercise lactate levels of 4.0 ± 0.7 $mmol \cdot L^{-1}$ following resistance training repetitions to failure at 60% 1RM, whereas the HIFT workout reported post-exercise lactate levels of 10.71 ± 2.35 $mmol \cdot L^{-1}$. Additionally, the vascular constriction elicited during the overhead squats may have counteracted the vasodilation effects of the nitrate supplementation reduction to NO (21). Even though the pace of the HIFT was at a moderate intensity there would have been a significant metabolic stress due to the exercise selection mitigating the beneficial properties of the dietary nitrate supplementation.

Additionally, the volume of nitrate consumed was relatively smaller compared to the previous HIIT trials. The current study utilized an acute dose (70 mL) of the standard, single serving, over-the-counter Beet It Sport (Ipswich, UK) supplement containing ~4.2 mmol NO_3^-. Previous HIIT studies (4, 6, 15, 16, 25) used significantly higher doses (> 6.4 mmol NO_3^-), with some using the supplement over multiple days (5-7 days). As such, the dosing and dose timing may have also played a role in not detecting a difference in HIFT performance following acute nitrate supplementation. Wylie et al (24) had determined that there is a pharmacodynamic and dose-response relationship to the consumption of various volumes of dietary nitrates (70 mL, 140 mL, 240 mL) with the blood concentration peaking at 2-3 hr post consumption and the higher volumes all yielded progressively higher blood concentrations, however, a single "shot" is sold in the studied 70 mL dose.

HIFT has become an increasingly popular training modality and as such more and more participants will look to supplements to improve performance (7). In order to see improvement in HIFT performances, which are often judged by reductions in time to completion or increasing the number of sets performed in a given time, with beetroot nitrate supplementation, the dosing may need to be increased beyond the standard, single-serving, over-the-counter 70 mL (~4.2 mmol NO_3^-) dose as well as possibly needed to be consumed over multiple days. However, it is still unclear if the nature of the HIFT modality, large volume sustained or high repetition muscle contractions, may play a role in blunting the nitrates effect on blood flow and metabolic stress compared to HIIT modalities which incorporate regular rest periods which allow for increased capillary bed reperfusion.

Beetroot nitrates, taken in a 70 mL dose, does not to improve HIFT performance. This could possibly be due to the combination of aerobic and anaerobic demands throughout the HIFT workout, particularly the vascular compression effects of the overhead squats and high metabolic demand of the activity. Previous research has demonstrated that dietary nitrate supplementation has an inconclusive effect on high-intensity exercise and that the single-serving 70 mL dose impact may be even less so. There may need to be higher dosing (> 70 mL) needed as well as regular consumption to be effective for HIFT (6).

Future research should examine the effect of different acute dosing on BR on HIFT training as well as the potential chronic BR effects. Additionally, the addition of VO_2 monitoring during the field tests would add a valuable piece to understand how oxygen cost is directly affected during HIFT with and without BR.

However, this study was not without limitation. The lack of VO_2 monitoring limited our ability to conclude how oxygen cost was affected with and without BR as well as our ability to determine participants aerobic . Furthermore, we also did not control diet for nitrate consumption nor did we limit the use of oral antibiotic mouth rinse that may have impacted overall nitrate supplementation.

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