



Original Research

The Effects of Beetroot Juice on VO₂max and Blood Pressure during Submaximal Exercise

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ABSTRACT

International Journal of Exercise Science 12(2): 332-342, 2019. Beetroot juice (BR) has been shown to reduce blood pressure (BP) at rest and improve several performance parameters during exercise. However, the effect of BR on BP during submaximal exercise has not been investigated and its effects on VO₂max are inconclusive. The purpose of this study was to investigate the effects of BR on VO₂max and BP during submaximal exercise. 20 healthy, recreationally trained volunteers (age 21.8±2.35 years, weight 75.10±10.62 kg, height 177.4±6.39 cm) participated in this study, which had a double-blind placebo controlled randomized crossover design. Participants supplemented with either 237 ml servings of placebo or 70 ml BR servings (nitrate concentration of 6.4 mmol/day) for 7 days. Participants completed a ramp treadmill protocol to determine VO₂max. BP was taken at 70% max heart rate calculated using the Karvonen method. There was no significant change in VO₂max after BR supplementation (51.07±6.12 ml/kg/min) versus placebo (50.46±6.06 ml/kg/min), $t(19)=1.41$, $p=0.17$. There was no significant change in either systolic BP after BR supplementation (180.65±23.37 mm Hg) versus placebo (177.65±22.07 mm Hg), $t(19)=0.49$, $p=0.63$, or in diastolic BP after BR (92.90±18.89 mm Hg) versus placebo (90.75±17.73 mm Hg), $t(19)=0.51$, $p=0.62$. BR did not affect VO₂max, nor did it affect BP during submaximal exercise.

KEY WORDS: Ergogenic aid, supplement, performance, nitric oxide, aerobic capacity

INTRODUCTION

The higher the VO₂max value produced by an individual, the greater the rate at which an athlete's body can consume oxygen, and the higher their level of cardiovascular fitness. Minor increases in VO₂max values can have remarkable advantages. For example, some researchers speculate that if every player on a soccer team has a VO₂max of just 6 ml/kg/min higher than their opponent, the aerobic capability possessed would equate to essentially having an additional player on the field (32). VO₂max can be increased in an individual by participating in different types of endurance training programs (22), but there may be a way to increase it using ergogenic supplemental aids.

A higher VO_2max corresponds to a greater delivery of oxygen from the blood to muscle tissue, so athletes may work at a higher intensity for a longer time. Increased blood flow is attributed mostly to increased cardiac output, but also to the muscle pump and local mediation of vasodilation near working muscle tissue. This occurs as a natural response to exercise and can increase blood flow exponentially compared to resting values (27, 29). Focusing on vasodilation, several sources (5, 7, 25) have suggested that nitric oxide (NO) is a prime solicitor in the process. NO is not ingested directly, rather it is derived from substances endogenously manufactured or ingested. NO is synthesized via at least two physiological pathways: NO synthase (NOS)-dependent and NOS-independent. Research pertaining to the NOS-dependent pathway does not appear to show any effects on VO_2max (28, 6, 4, 1, 10). Thus, further research on the effects of NO on VO_2max should shift its focus on investigating the NOS-independent pathway instead.

Research on supplementation through the NOS-independent pathway involves sodium nitrate and beetroot. In a study by Larsen et al. (18), supplementation with sodium nitrate (0.1 mmol/kg of body mass/day for 3 days) in moderately trained subjects showed a decrease in VO_2 at submaximal intensities, indicating a beneficial reduced oxygen cost during exercise. Gross efficiency, which was defined as work rate divided by energy expenditure, was also significantly improved. A later study by Larsen et al. (19) on moderately trained subjects used the same loading scheme for 2 days and also showed a significantly lower VO_2peak after sodium nitrate ingestion without any effect on time to exhaustion. A study done on well-trained subjects showed a similar significantly reduced VO_2peak compared to placebo, but with no effects on VO_2 at submaximal intensities. The dosage in this study was more acute (3 hours pre-exercise) compared to previous studies and used 10 mg/kg of body mass of sodium nitrate (3).

In a study done on physically active individuals, beetroot supplementation for 15 days showed a significant increase in peak power and VO_2max . Additionally, steady-state VO_2 at moderate-intensity exercise was significantly reduced 2.5 hours after ingestion (30). In other studies, it has been shown to increase power output (16), delay time to exhaustion, and increase efficiency not only at the onset of exercise, but also in moderate (80% of gas exchange threshold) and severe intensity exercise (70% of the difference between the power output at gas exchange threshold and VO_2peak) (2, 17).

Nitric oxide supplements have been shown to increase mitochondrial efficiency by improving the amount of oxygen reduced per ATP produced, otherwise known as the mitochondrial P/O ratio (20). Theoretically, this would explain the suggested benefits that previous studies have shown on the cardiorespiratory components of aerobic exercise. Keeping this in consideration, along with all the aforementioned results, it could be hypothesized that a nitric oxide supplement would help increase VO_2max . With respect to the NOS-independent pathway, results are contradicting between two forms of NO donor. There are two studies using sodium nitrate that showed a significantly reduced VO_2peak and VO_2max following supplementation (3, 19), while there is one study using beetroot juice that showed an increase in VO_2max (30). Furthermore, the study that used beetroot juice used a ramp cycle ergometer test to gather VO_2

data, while it has been shown that an individualized ramp treadmill protocol elicits higher, more valid values and is more likely to bring an individual to a true VO_2max due to the increased volume of muscle mass used (12, 24). In addition, it is widely accepted that typical blood pressure responses during incremental cardiorespiratory endurance exercise include an increase in systolic pressure and a maintenance of diastolic pressure. It is also known that NO is effective at reducing blood pressure at rest (2, 17, 30). However, there appear to be no studies that have investigated the effect of supplementation on blood pressure values during exercise. The purpose of this study was to investigate the effects beetroot juice on VO_2max and on blood pressure during submaximal exercise in recreationally trained, college-aged males.

METHODS

Participants

Twenty-one apparently healthy recreationally trained college-aged males who participate in at least 150 minutes of exercise per week were recruited, however only 20 completed the study (Table 1). Inclusion criteria included non-smokers and no supplement use for at least one month prior to the study. The study was approved by an Institutional Review Board for use of human subjects and all participants were informed of the risks and benefits of the investigation as well as their confidentiality prior to signing an informed consent form and completing a PAR-Q+ health status questionnaire. Participants were asked not to change their diet and to complete a three-day food log for the three days before each testing session. Before testing, all participants were asked to avoid caffeine for 6 hours, eating for 2-3 hours, and alcohol and exercise for 24 hours. All testing was conducted at the same time of day during each session in the Hanner Human Performance lab.

Table 1. Participant descriptive information.

	Age (years)	Height (cm)	Weight (kg)	Target Heart Rate (bpm)	Age-Predicted Max Heart Rate (bpm)
n=20	21.8±2.35	177.4±6.39	75.10±10.62	158.5±3.40	198±2.35

Protocol

Participants were required to report to the laboratory on three occasions. During the first session, participants were instructed and familiarized with the VO_2max treadmill ramp protocol. This protocol has been validated as a more suitable alternative compared to cycle ergometry and other methods for predicting oxygen uptake due to its gradual and individualized increases in work (24). Additionally, participants were instructed and familiarized with the Finapres Pro system and how to dismount the treadmill during the VO_2max test for the blood pressure reading. Finally, baseline descriptive data were collected (height, weight, resting heart rate) and participants were given either a week's worth (7 days) of 237 ml servings of placebo (water with McCormick red food coloring FD&C reds 40 and 3) or a week's worth of 70 ml beetroot juice servings (Beet it, James White Drinks, Ipswich, UK, nitrate concentration of 6.4 mmol/day). This duration was chosen because it closely resembles what was used in previous studies investigating changes in oxygen consumption (2, 16, 17, 30). These were randomly assigned by a third-party who was unrelated to the study. These

servings were conveniently measured as individual bottles, so the participants needed only to consume one bottle per day. Participants were asked to consume the treatment at the same time of day for 7 days. After the week concluded, the participants returned to the laboratory and performed a VO₂max test and data were recorded. Participants were then given a week's worth of the remaining treatment that they did not receive the first time and were instructed not to begin consuming for three days. This was to ensure that a 10 day washout period for the first treatment would transpire before the next testing session. A 10 day washout period was selected due to its use in previous studies, and includes the 7 day loading phase of the remaining treatment (2, 16, 17, 30). Participants then began ingestion of the given treatment every day for 7 days just like before. Following the second round of treatment, participants returned to the lab and completed the final VO₂max test and data were recorded. In order to increase compliance, all participants received daily text reminders to take their supplement. In addition, they were reminded when to report to the lab. VO₂max tests were conducted on a calibrated 4Front Woodway treadmill (PRO 27, Waukesha, WI, USA) using a ParvoMedics TruOne metabolic system (2400, Sandy, UT, USA) computer program using indirect calorimetry to record 15-second gas analysis averages. Heart rate was monitored using a Polar Ft1 heart rate monitor (Lake Success, NY, USA) and chest strap. This study utilized a modified ramp protocol based on the methodology of McCormack et al. (21) and La Monica et al. (15). The participants warmed up for five minutes at a self-selected comfortable speed at 0% grade before each test. Once the test began, the participants progressed through four, two minute stages in which the speed increased by 0.8 km/h each stage building off of the self-selected warm up speed. The fifth and consequent stages were 60 seconds long. The speed remained constant, but the grade began to increase by 2% every minute until volitional exhaustion. A VO₂max value was accepted if at least 2 of the following criteria were met: Rate of perceived exertion (RPE) of 18-20, heart rate within 10 beats of age-predicted maximum, plateau in the VO₂ curve, Respiratory exchange ratio (RER) greater than or equal to 1.15. These values have been validated as marker criteria for VO₂max (24).

Age-predicted max heart rate was used in the Karvonen formula to calculate a target heart rate at an intensity of 70%. The blood pressure data were collected using the Finapres Pro system when participants reached this target heart rate during the VO₂max test, which typically occurred about three to four minutes into the test. This system was fitted on the participants prior to beginning the warm-up, with a Velcro band that enveloped the proximal portion of one arm, so that it applied pressure to the brachial artery. Another band was fitted around the wrist of the same arm and a third band around the middle digit of the hand. The structure and tightness of these bands ensured that the small tubes and wires that were attached to the device were stabilized. The most proximal band was used to measure blood pressure (BP) in the brachial artery. This arm cuff inflates and then gradually deflates and is similar to a typical BP monitor found in a doctor's office. The band around the finger however, measures BP continuously. The computer program regulates the pressure applied around the finger so that the cuff remains snug, but does not apply so much pressure that it will cause pain or significant obstruction of blood flow. Due to the sensitivity of the system, participants were asked to straddle the treadmill, ungrasp the safety rail with the measurement hand, and momentarily hold the arm freely at heart level across the chest for the measurement to be

taken. Immediately after the measurement was taken, the Finapres Pro system equipment was removed and participants were instructed to safely remount the treadmill and continue the VO₂max test.

Statistical Analysis

To test for differences in the dependent measures (VO₂max, submaximal intensity exercise blood pressure), all recurring measures were analyzed using a paired samples t-tests using the statistical package for social sciences (SPSS v.23). It was assumed that the dependent variables were measured on a continuous scale, that the independent variable consisted of 2 related groups, that there would be no significant outliers, and that differences in the dependent variables between the 2 groups would be normally distributed. With respect to α level, significance was considered at $p \leq 0.05$. Effect size was calculated using Cohen's D. Participants acted as their own control.

RESULTS

All 20 participants who completed the study reported a 100% adherence to the supplementation dosing protocol for both treatment periods and also reported maintenance of their regular physical activity and exercise habits. Participants routinely reported the passing of red or pink urine, which could be attributed to the betalain pigments in beetroot juice or the red food coloring, but only one participant was removed due to stomach discomfort. No participants were excluded after reviewing the 3-day food recalls for dietary inconsistencies between the two supplementation periods. 12 participants began with the beetroot juice treatment and 8 began with the placebo. All participants fulfilled either the direct or indirect criteria for reaching a true VO₂max.

After beetroot juice supplementation compared to placebo there was no significant difference in VO₂max values, $t(19)=1.41$, $p=0.17$, in respiratory exchange ratio (RER) values at VO₂max, $t(19)=1.59$, $p=0.13$, in systolic blood pressure, $t(19)=0.49$, $p=0.63$, nor in diastolic blood pressure, $t(19)=0.51$, $p=0.62$ (Table 2).

Table 2. Variables following both treatments.

	VO ₂ max (ml/kg/min)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	RER	RPE
Beetroot Juice (BR)	51.07±6.12	180.65±23.37	92.90±18.89	1.15±0.05	18.9±0.72
Placebo (PL)	50.46±6.06	177.65±22.07	90.75±17.73	1.13±0.07	19.0±0.65

VO₂max, maximal oxygen consumption; Systolic BP, systolic blood pressure; Diastolic BP, diastolic blood pressure; RER, respiratory exchange ratio; RPE, rate of perceived exertion.

DISCUSSION

Nitrates, like those found in beetroot juice, have garnered interest due to their effects on lowering blood pressure at rest (2, 17, 30), however there is also a line of research investigating their effects on performance. The current literature regarding the ergogenic effects of nitrates is mostly concentrated on four different NO donors: L-arginine, L-citrulline, sodium nitrate, and

beetroot juice. These NO donors are categorized into two groups, based on whether they are oxidized in the NOS-dependent physiological pathway, or reduced in the NOS independent physiological pathway. L-arginine and L-citrulline pertain to the former, while sodium nitrate and beetroot juice pertain to the latter. The purpose of this study was to investigate the effects of beetroot juice compared to placebo on VO_2 max and blood pressure during submaximal exercise on recreationally trained college-aged males. VO_2 max, RER, systolic blood pressure, and diastolic blood pressure were analyzed for differences between the supplement and the placebo. There were no significant differences in any of these variables after beetroot juice supplementation compared to placebo.

NOS-dependent NO donors have consistently shown to not affect VO_2 max (28, 6, 4, 1, 10). More relevant to this study, the NOS-independent donors have shown mixed results. Sodium nitrate decreased VO_2 peak in two studies (3,19), while beetroot juice increased VO_2 max in one study (30) and did not affect VO_2 peak or VO_2 max in three others (2, 16, 17).

The dosing protocol in the present study used supplementation periods of 7 days in order to emulate the previous studies that investigated other aspects of performance (2, 16, 17). Of the 4 studies involving beetroot juice, only one reported a significant increase in VO_2 max (30). The purpose of that study was to investigate whether differences in physiological responses to moderate-intensity and incremental exercise existed among different durations of supplementation. That study used a slightly different product compared to the present study, but from the same company (Beet it, James White Drinks, Ipswich, UK). Rather than use the 70 mL servings containing approximately 6.4 mmol of nitrate, these researchers used a 0.5l/day serving with a concentration of approximately 5.2 mmol of nitrate. Comparisons were made between values attained 2.5 hours, 5 days, and 15 days after supplementation. When compared to baseline values, VO_2 max was significantly higher in the beetroot juice group, but not the placebo group after 15 days of supplementation. One could speculate that perhaps no differences were seen in the present study because the duration of supplementation was not long enough, however despite being different in dosage duration and perhaps dose concentration, the 2 studies also differed in number of participants and exercise protocol. The present study had 20 male participants compared to 8 total comprised of 5 males and 3 females. The present study also used an individualized treadmill ramp protocol rather than a cycle protocol due to the former being shown to be more likely to elicit higher and more valid VO_2 values compared to the latter (12, 24). Although there were no significant differences in VO_2 max between treatments in the present study, previously, differences between groups could only be speculated to be facilitated by the nitrate content in beetroot rather than by other compounds like betaine, antioxidants, and polyphenols which have been shown to potentially increase performance variables like exercise tolerance, power, and mitochondrial function (11, 8, 14). The use of a nitrate-depleted beetroot juice as the placebo by Lansley et al. (16) and Lansley et al. (17) helped to strengthen the argument that any differences were largely attributed to the nitrate content in beetroot juice rather than the above mentioned compounds.

In addition to VO_2max , RER was also not significantly different after beetroot juice supplementation. This finding lends further support to Bailey et al. (2) and Lansley et al. (17), who reasoned that the similarity indicated there were no differences in substrate utilization, which would have an impact on the oxygen cost of the task. Given no significance, it is important to note that efficiency or oxygen cost was improved in other studies (18, 20, 17, 2) and there is a possibility that this occurred in the present study, but was not measured. Collection of data regarding time to exhaustion would help make conclusions regarding that speculation. Unfortunately, data regarding time to exhaustion were not collected due to the inconsistency of time required to remove the Finapres Pro system equipment from participants during the VO_2max test.

At rest, beetroot juice has been shown to decrease resting systolic blood pressure (17) as well as diastolic blood pressure (30, 31). Systolic and diastolic blood pressure at 70% of max heart rate calculated using the Karvonen formula did not show a significant change after beetroot juice supplementation. A study by Miyai et al. (23) investigated the blood pressure response to heart rate during exercise in 1033 normotensive men. The blood pressure mean values at 70% max heart rate from the present study were comparable to those found the study. The diastolic blood pressure means from the present study fell into the 50th percentile values of those 1033 men at 70% of heart rate reserve, while the systolic blood pressure means fell into the 25th percentile.

The present study had a few limitations. First, with this study design, there is a possibility that a testing effect can occur between the 2 testing days. Most participants had never experienced a VO_2max test and thus were possibly better prepared when performing their second test, as they knew what to expect. It is possible that one who is unfamiliar with maximal testing may terminate his or her first test a tad early, but withstand slightly longer during the second session now that he or she better understands his or her physical capacity or limits. This realization could have produced slight differences in final VO_2max values, despite all participants fulfilling either the direct or indirect criteria for accepting a VO_2max value. In order to avoid this effect, all efforts were made to ensure that 10 participants began with the placebo and that 10 began with the beetroot juice. Unfortunately, because of unforeseen participant dropout, 12 participants began with the beetroot juice treatment and 8 began with the placebo.

Second, the taste of beetroot juice is not easily matched or replicated. Participants noted the strong taste of one of the treatments, presumably the beetroot juice, which could have made it easier for the participants to decipher what treatment they were taking. The strong taste difference made this especially true if participants were randomly assigned the beetroot juice as the first treatment. Although every effort was made to avoid and discourage discussion of the treatment, a few participants still stated their suspicion of the placebo contents.

Third, the sensitivity of the Finapres Pro system, although helpful, was also problematic. The movement during exercise caused the readings of blood pressure on the system's screen to oscillate tremendously second-by-second. For this reason, participants were asked to quickly

temporarily straddle the treadmill to get a reading while the data collection arm was motionless. Immediately upon the cessation of exercise, blood pressure begins to decrease back to resting values, so recording a value quickly is pertinent. During the actual straddling of the treadmill, blood pressure had a tendency to fluctuate wildly in most participants because of the movement of the data collection arm and the temporary gripping of the safety rails. The values quickly became more consistent within 2-3 seconds, which is when all readings were taken, and remained so for at least several seconds before beginning to drop, which allowed for more accurate measurements.

Next, many participants experienced malfunctions with the Polar heart rate monitors. After some time during the tests, the monitors would stop recording heart rate data or produce irrational readings given the current intensity. Because this issue occurred on most occasions, it interfered when considering whether or not participants had reached the indirect criteria for accepting a VO_2 max value with respect to heart rate. The Finapres Pro system also records heart rate, so this was only an issue towards the end of testing when it was no longer equipped.

Another limitation was that the 3-day food diaries were not analyzed using a food log analysis software. Using this kind of software would allow more confident conclusions regarding the consistency and nitrate consumption of each participant's diet between the 2 testing sessions. Currently, the food log entries were evaluated to ensure that participants were eating regularly and similar amounts of similar meals across testing dates.

There also was not a standardized length of time between each final treatment and the subsequent VO_2 max trial. Participants were simply told to make sure at least 2 hours separated their last treatment ingestion and their testing session. Studies involving different dosing techniques using Beet It have shown that plasma nitrite concentration was significantly increased 2.5 hours post-ingestion (16, 30) and remained close to peak values 5 hours post-ingestion (31). This acute dose was shown to improve oxygen cost of moderate intensity-exercise (30), time to completion of a cycling timed trial, and power output (16). On the other hand, VO_2 max seems to only be affected after chronic supplementation (30). Although the majority of participants did complete their test at most 5 hours post-ingestion, a few simply could not due to conflicting university class schedules. The present study would have been strengthened if a standardized time between final treatment and testing trial could have been employed for all participants to avoid potential discrepancies in plasma nitrate levels that could have possibly translated to effects on performance.

Finally, blood samples were not drawn in this study to measure plasma nitrate/nitrite. This was a limitation mentioned in several other studies (9, 13, 26). If any significant differences did exist in the present study, it would have been difficult to attribute them to the nitrate content of the supplement. Other studies using very similar dosing protocols have counteracted this by using a nitrate-depleted placebo that is nearly identical to the supplement (16, 17).

Future studies investigating the effects of beetroot juice on VO₂max should consider employing a familiarization session in order to lessen the possibility of differences in data collection sessions due to inexperience with maximal testing intensities. Additionally, if the purchase of nitrate-depleted beetroot juice is available for future investigations and there is sufficient funding, its acquisition would be well worth the price in order to properly disguise the placebo. Finally, if blood pressure during exercise is to be measured in future studies, especially with equipment as accurate and with as high a sampling rate as the Finapres Pro system, researchers would be advised to perhaps use a cycling protocol which will allow minimal movement of the data collection arm for measurements with more accuracy and less noise.

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