

Effects of Acute Beetroot Juice Consumption on Physiological and Performance Variables during Endurance Exercise in Severe Hypoxia

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Abstract

Purpose: Acute consumption of red beetroot juice has been shown to reduce systemic blood pressure and improve endurance exercise performance. Inhalation or infusion of nitrates, the active ingredient in beetroot juice, has been shown to partially alleviate hypoxic pulmonary vasoconstriction (HPV) and hypoxic pulmonary hypertension, and partially restore exercise performance in severe hypoxia. We studied the effects of acute red beetroot ingestion on physiological markers of HPV and performance during high-intensity endurance cycle ergometry in severe hypoxia.

Methods: Nine male competitive cyclists completed a performance time trial (PTT) at a PP_{O_2} of 86 Torr following acute ingestion of red beetroot juice (BR), denitrated beetroot juice (PL), or water. Systemic blood pressure and arterial oxygen saturation (SpO_2) were measured prior to and during exercise. Oxygen consumption was measured during exercise. Time to complete the PTT and average power output were used as performance markers and exercise economy was calculated based on exercising oxygen consumptions and power outputs.

Results: No significant differences were observed due to treatment for any of the physiological or performance variables.

Conclusion: Results suggest that acute beetroot juice consumption does not affect performance or physiological markers of HPV during exercise in severe hypoxia.

Keywords: Beetroot Juice, Hypoxia, Cycle Ergometry, Time Trial Performance, Hypoxic Pulmonary Vasoconstriction

1. INTRODUCTION

When humans are exposed to hypoxic environments, the lowered atmospheric oxygen pressure results in a reduction in the oxygen pressure gradient between the alveoli and the blood in the pulmonary circulation bed. As a result of the lowered pressure gradients, blood oxygenation (SaO_2) levels drop which, consequently, results in lower oxygen delivery to working muscle, oxygen consumption and endurance exercise performance. As ambient oxygen pressure drops below 90 Torr, widespread constriction of the pulmonary vasculature occurs [1,2]. This condition, known as hypoxic pulmonary vasoconstriction (HPV), further contributes to reductions in the transfer of oxygen from the alveoli to the blood by reducing the interface area between these two compartments. As a result, further reductions in SaO_2 levels, aerobic metabolism, and endurance

exercise performance occurs [3,4]. While the physiological mechanisms leading to HPV have not been fully elucidated, it appears to be at least partially related to cyclic GMP degradation, as HPV has been somewhat alleviated by the use of PDE-5 inhibitors [5,6,7] and inhaled or infused nitrites and nitric oxide [8,9] in PP_{O_2} environments ranging from 70 – 90 Torr. Interventions with PDE-5 inhibitors at these oxygen pressures have also been shown to partially restore SaO_2 , oxygen consumption, and performance in exercising subjects who are experiencing HPV [5,6,7].

Acute and chronic consumption of dietary nitrates via red beetroot juice (RBJ) have been shown to increase nitric oxide availability, promote systemic vasodilation, reduce systemic blood pressure, and improve performance and economy during exercise in normoxic environments [10,11,12]. These observations,

and those that have utilized inhaled or infused nitrates [8,9], suggest that oral RBJ consumption may be able to moderate HPV and partially restore performance, oxygen consumption, and SaO₂ during exercise in severe hypoxia. Some investigations [13,14,15,16,17] have studied the physiological and performance responses to RBJ consumption during exercise in hypoxia (PP_{O₂} = 100 - 120 Torr) and have had mixed results. However, other investigations [18,19,20] suggest that these levels of hypoxia may have been inadequate to promote HPV and thus, HPV related changes would not have been observed as a result of BRJ consumption. Thus, we sought to determine the effects of acute BRJ consumption on resting systemic blood pressures, resting and exercising blood arterial oxygen saturations, oxygen consumption, performance, and economy during cycle ergometry in severe hypoxia (PP_{O₂} = 86 Torr).

2. METHODS

Prior to subject recruitment, the procedures of this investigation were reviewed and approved by the institutional review board of Appalachian State University. All data were collected at the Vascular Biology and Autonomic Studies Laboratory of Appalachian State University at an ambient barometric pressure of approximately 665 Torr. Nine male cyclists with at least one year of competitive experience in time trialing served as subjects for this investigation. Vital characteristics for these subjects were (mean ± SD) 22 ± 4 yr, 181 ± 8 cm, 73.8 ± 0.8 kg, with a VO₂ max of 66.7 ± 8.6 mL/kg/min. Each subject was required to signify willingness to participate by reading and signing a written informed consent. All subjects had lived between approximately 1000 and 1300 m. for the previous six months. Occasional travel and competition excursions outside of these altitudes did occur during this time period; however, individuals who made extended sojourns (more than 2 days) outside of these levels were excluded.

3. STUDY OVERVIEW

During the initial laboratory visit, subjects performed a graded exercise test to exhaustion to determine VO₂ max followed by a practice trial of a performance time trial test (PTT) that was to be performed on subsequent visits. Subjects returned to the laboratory on three separate occasions to perform the PTT under hypoxic conditions following acute

consumption of one of three separate treatments: red beetroot juice (BR), de-nitrated red beetroot juice (PL), or water (NT), each provided in equal volumes. All exercise testing was performed using a Lode Excalibur Sport electronically braked cycle ergometer (Lode, Groningen, Netherlands) adjusted to duplicate the dimensions of each subject's bicycle.

3.1. Determination of VO₂ max and Practice Performance Time Trial

Maximal oxygen consumption was assessed using a previously presented protocol [21]. Following a standard warm-up, subjects began the test at a work rate of 3 W/kg bm and progressed by 0.3 W/kg bm/min until exhaustion. Expired air was collected and analyzed using a Parvomedics Trueone 2400 metabolic cart (Parvomedics, Sandy, UT) calibrated to manufacturer's specifications. Criteria for a maximal effort included volitional exhaustion, maximal heart rate similar to estimated (220 - age), and a RER of greater than 1.15. Upon completion of the maximal test, subjects were allowed 20 min of recovery before performing the practice trial of the PTT. No data was collected from the practice PTT and this trial was used to familiarize the subject with the equipment and the requirements of the trial. These trials were performed under ambient conditions (F₁O₂ = 20.93%, barometric pressure ~ 665 Torr, PP_{O₂} ~ 140 Torr, temperature = 21-23° C).

3.2. Experimental Trials

Subjects returned on three occasions to perform the PTT in hypoxia (F₁O₂ = 13%, PP_{O₂} = 86 Torr) following consumption of the experimental treatments. Two hours prior to arrival, subjects consumed a standardized 460 kcal meal consisting of two energy bars (Clif Bar & Company, Emeryville, CA) and one liter of water. Upon arrival, subjects were placed in a seated position where they rested quietly for 15 min. Following this rest period, subjects remained seated while systemic blood pressure was measured at the brachial artery using the first and fifth Korotkoff sounds. All blood pressure measurements were taken by the same technician using the same sphygmomanometer and stethoscope. The subjects then consumed 70 mL of red beetroot juice with approximately 6.45 mMol nitrate (BR), 70 mL of nitrate depleted beetroot juice with approximately 0.05 mMol of nitrate (PL) (James White Drinks, Ipswich, UK), or 70 mL of tap water (NT). This

BRJ dose strategy has previously been shown to significantly increase plasma nitrate levels and influence physiological and performance variables in exercising individuals [14]. The treatments were applied in a randomized, double-blind, crossover design.

After consuming the treatment, subjects rested quietly for 135 min before a second systemic blood pressure measurement was taken as previously described. One hundred forty minutes after consumption of the experimental treatments, subjects mounted the ergometer and began a standardized 10-min warm-up. The warm-up began at 50 W below the starting work rate for the subject's VO_2 max test and progressed by 10 W/min for five minutes. The subjects then maintained this work rate for the remaining five minutes. Five minutes following the conclusion of the warm-up, a third systemic blood pressure measurement was taken while the subjects remained seated and motionless on the ergometer. Following the blood pressure measurements, the subjects remained on the ergometer and were introduced to the hypoxic gas. The subjects then breathed the hypoxic gas while resting quietly on the ergometer for three minutes prior to the beginning of the PTT. Subjects continued to breathe the hypoxic mixture for the duration of the PTT.

3.3. Performance Time Trial

The PTT consisted of 2.5 kJ/kg bm of work performed as quickly as possible. Prior to the start of the PTT, the ergometer was placed in the manual (cadence independent) mode which allowed subjects to manually adjust the power output through the use of toggle switches. Subjects began the PTT from a standing start at an initial work rate of 3 W/kg bm. During the PTT, elapsed work was continuously displayed to the subjects and they were allowed to adjust the work rate at their discretion; however, the subjects were blinded to their work rates and elapsed time. Subjects received vigorous, positive, verbal encouragement during each of the experimental PTTs by the same two investigators. Time to complete the PTT was measured to the nearest second using a digital stopwatch. Expired air was collected throughout the PTT and analyzed for oxygen consumption. The metabolic cart was calibrated to manufacturer's specifications for hypoxic conditions using hypoxic gas from the same cylinder that was used to supply air to the exercising subject during the PTT. SpO_2 ,

measured by earlobe pulse oximetry (Nonin 8500, Nonin Medical, Plymouth, MN), was recorded at rest just prior to the start of the PTT, as the subjects completed 25, 50, and 75% of the total work, and just prior to the completion of the PTT. A minimum of 48 hr and a maximum of 7 d were allowed between each PTT and this interval was kept consistent for each individual subject. Each subject performed their three PTTs at similar times of day.

4. DETERMINATION OF EXERCISE ECONOMY

Oxygen consumption was measured throughout the PTT and expressed in mL/kg bm/min. From these values, an average oxygen consumption rate was determined for each of the three experimental conditions. The average oxygen consumption values from each of the three PTT conditions were then divided by their respective average power outputs to calculate exercise economy expressed as mL of O_2 /kg bm/min/W for each of the three conditions.

5. APPLICATION OF HYPOXIC GAS

Gases were administered to the subjects by a device described elsewhere [22]. Briefly, medical grade gases (Airgas, Hickory, NC) were administered from gravimetrically determined cylinders into a 120 L reservoir. The subjects breathed the gas from the reservoir through a Hans Rudolph model 2700 two-way, non-rebreathing valve. To avoid slight variations in gas mixtures between cylinders, each individual subject breathed gas from the same cylinder for each of his PTTs.

6. LIFESTYLE REQUIREMENTS

Subjects were restricted from habits that may have affected physiological responses to experimental treatments and performance of experimental protocols. Each subject maintained similar diets and exercise schedules beginning one week prior to participation until the final experimental protocol was completed. Diets were restricted from high-nitrate and antioxidant foods and supplements. Caffeine and alcohol consumption were prohibited for 24 hr and chewing gum and mouthwash were prohibited for 48 hr prior to each visit. Subjects were required to consume one liter of water the night before and the morning of, each visit. With the exception of the standardized snack consumed two hr prior to each visit, the subjects were four hr post-prandial for each visit. Activity restrictions included no more than 30 min of light exercise ($\leq 60\%$ of maximum heart

rate) on the day prior to each visit and no exercise on the day of each visit. Subjects were required to maintain and present food consumption and activity logs throughout their participation.

7. STATISTICAL MEASURES

All statistical analyses were performed using SPSS version 20 (IBM, Armonk, NY). Time trial performance, oxygen consumption, and exercise economy were analyzed using separate one-way, repeated measures ANOVA. All other dependent variables were analyzed using a two-way repeated measures ANOVA. All data sets were tested for normality using a Shapiro-Wilk test. A post-hoc test utilizing Sidak correction was applied when appropriate. All values are reported as means ± standard deviations unless noted otherwise. Level of significance was set *a-priori* at $P \leq 0.05$.

8. RESULTS

Exposure to hypoxia and all treatments were well tolerated by all subjects with no harmful side-effects being reported by the subjects or

observed by the investigators. Written records suggested that all subjects complied with lifestyle requirements. Shapiro-Wilk results suggested that all data sets were normally distributed with P values ranging from 0.47 to 0.92.

8.1. Blood Pressure

Systolic, diastolic, and mean arterial blood pressures were measured at three time points: 1) prior to ingestion of treatments (PreI), 2) 135 min after ingestion of the treatments (immediately before the warmup) (PreWU), and 3) 155 min after the ingestion of the treatments (five min after the warmup) (PostWU). Significant effects due to time were observed in systolic and mean arterial blood pressures but no significant difference were detected due to treatment. No other differences in blood pressure measurements were observed due to time or treatment. Blood pressure data are presented in Table One.

Table1. Blood Pressure Response

	Treatment		
	NT	PL	BR
Systolic (mm Hg)			
PreI	118 ± 8	117 ± 4	123 ± 7
PreWU	117 ± 9	113 ± 8	117 ± 9
PostWU	125 ± 10	125 ± 7 [#]	128 ± 7 [#]
% Change PreI vs PreWU	-2 ± 5	-3 ± 5	-5 ± 5
% Change PreI vs PostWU	6 ± 7	7 ± 5	4 ± 7
Diastolic (mm Hg)			
PreI	76 ± 8	78 ± 9	74 ± 7
PreWU	79 ± 6	79 ± 9	78 ± 6
PostWU	80 ± 8	79 ± 12	80 ± 6
% Change PreI vs PreWU	4 ± 9	1 ± 5	5 ± 11
% Change PreI vs PostWU	6 ± 16	1 ± 11	14 ± 14
Mean Arterial (mm Hg)			
PreI	90 ± 6	91 ± 5	90 ± 6
PreWU	91 ± 5	90 ± 8	91 ± 6
PostWU	95 ± 6	95 ± 8	98 ± 6 [#]
% Change PreI vs PreWU	1 ± 3	-1 ± 5	1 ± 6
% Change PreI vs PostWU	6 ± 9	4 ± 6	9 ± 10

BR = Beetroot Juice

PL = Placebo

NT = No Treatment

PreI = Pre-Ingestion of Treatment

PreWU = Pre-Warmup (135 min Post-Ingestion of Treatment)

PostWU = Post-Warmup (155 min Post-Ingestion of Treatment)

* $P < 0.05$ Compared to PreI for that Treatment

[#] $P < 0.05$ Compared to PreWU for that Treatment

8.2. SpO₂, Oxygen Consumption, and Exercise Economy

Resting SpO₂ was measured just prior to the start of the PTTs. Exercising SpO₂ during the PTTs was collected as subjects completed 25, 50, and 75% of the total work and just prior to

the completion of the trials. No differences in SpO₂ were seen between the different exercising time points; therefore, exercising data from each of the three experimental trials were combined to calculate an average exercising SpO₂ for each trial. In each of the three treatments, resting SpO₂ was significantly higher when compared to exercising SpO₂ (NT: Rest = 94 ± 4%, Exercise = 74 ± 3%, P < 0.01; PL: Rest = 94 ± 3%, Exercise = 74 ± 5%, P < 0.01; BR: Rest = 94 ± 2%, Exercise = 76 ± 4%, P < 0.01); however, treatment had no effect on resting (P = 0.99) or exercising (P = 0.23 – 0.94) SpO₂. Average oxygen consumptions during the PTTs were 30.99 ± 4.13, 30.30 ± 3.24, and 30.76 ± 2.27 ml/kg/min for BR, PL, and NT, respectively (P = 0.79 – 0.99). No significant differences between treatments were observed in economy (NT = 0.15 ± 0.01 mL O₂/kg bm/min/W, PL = 0.14 ± 0.02 mL O₂/kg bm/min/W, BR = 0.15 ± 0.02 mL O₂/kg bm/min/W) with P values ranging from 0.77 to 0.99.

8.3. Exercise Performance

There were no significant differences between treatments in the time to complete the PTTs (BR vs PL, P = 0.10; BR vs NT, P = 0.49; PL vs NT, P = 0.91). Time to completion data for the PTTs are presented in Figure 1. The percent differences in the time to complete the PTT for BR vs NT (+ 2.32 ± 10.57%) and PL vs NT (- 2.43 ± 5.42%) also revealed no significant effect of treatment on exercise performance (P = 0.12). No significant differences (P = 0.10 – 0.91) were observed in the average power outputs during the three trials (213 ± 20 W (NT), 215 ± 17 W (PL) and 208 ± 17 W (BR)). The PTT data were analyzed for an order effect. Average completion time for PTTs one through three were 897 ± 154 sec, 873 ± 141 sec, and 895 ± 146 sec, respectively (1 vs 2, P = 0.83; 1 vs 3, P > 0.99; 2 vs 3, P = 0.35) suggesting that no order effect occurred.

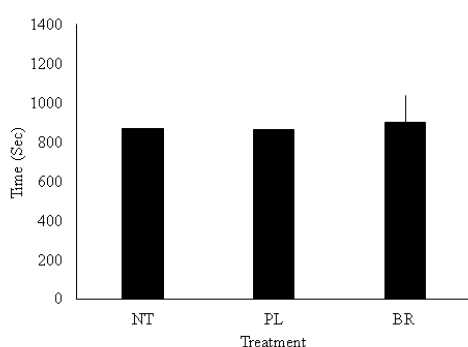


Figure 1. Mean Time to Complete the Performance Time Trial.

NT = No Treatment

PL = Placebo

BR = Beetroot Juice

9. DISCUSSION

To our knowledge, this is the first investigation of the effects of acute beetroot juice supplementation on time trial performance and physiological variables during exercise in hypoxia of sufficient level to promote HPV. No effects of beetroot juice supplementation were found with respect to resting systemic blood pressures, resting or exercising SpO₂, exercising oxygen consumption rates, exercise economy, or exercise performance.

Blood oxygen levels and vigorous endurance exercise performance typically decrease in response to hypoxia. In moderate hypoxia, the primary cause of arterial desaturation is the reduced oxygen pressure in the alveoli and lower oxygen pressure gradient between the alveoli and the blood of the pulmonary vasculature. As hypoxia becomes more severe, pulmonary vascular smooth muscle begins to contract and HPV becomes a contributing factor to arterial desaturation [23]. Previous works using PDE-5 inhibitors [5,6,7], inhaled nitric oxide [8] or infused nitrites [9], have demonstrated that these agents can partially alleviate HPV, resulting in partial restoration of blood oxygen saturation, VO₂ max, and exercise performance in severe (PP_{O₂} = 70 – 88 Torr) hypoxia. While no direct measures of HPV were made in the current study, no effects of BRJ were observed on resting or exercising SpO₂, exercising oxygen consumption, or exercise performance at a PP_{O₂} of 86 Torr suggesting that BRJ had no measurable effect on HPV.

Previous investigations have studied the effects of BRJ consumption on performance and physiological responses during exercise in hypoxia and have provided mixed results. Vanhatalo et al. [13] compared time to exhaustion during knee extension exercise in subjects under control conditions (PP_{O₂} ~ 159 Torr) and under hypoxia (PP_{O₂} ~ 110 Torr) with and without BRJ supplementation. Compared to control, time to exhaustion was significantly reduced from 471 sec. to 393 sec. when subjects exercised in hypoxia without BRJ. In contrast, consumption of BRJ prior to exercise in hypoxia

resulted in exercise performance that was almost identical (477 sec.) to control conditions. Muggerridge et al. [14] had subjects exercise at a PP_{O_2} of approximately 120 Torr after an acute dose of BRJ or placebo and noted improved exercise performance under the BRJ condition, but these investigators did not measure SpO_2 . Conversely, MacLeod et al. [17] used an identical BRJ supplementation strategy and similar ($PP_{O_2} \sim 120$ Torr) environmental conditions and exercise challenge compared to Muggerridge et al. [14] and saw no improvement in exercise performance or SpO_2 when compared to placebo. Kelly et al. [15], also saw no difference in exercising SpO_2 or performance in subjects who exercised at a PP_{O_2} of approximately 100 Torr following two days of supplementing with BRJ or placebo. Finally, Arnold et al. [16] did not measure SpO_2 , but saw no improvement in performance due to acute BRJ supplementation during exercise at a PP_{O_2} of approximately 100 Torr. However, each of these investigations were conducted at PP_{O_2} s ranging from 100 – 120 Torr which may not be adequate to promote HPV. Previous investigations using PDE-5 inhibitors suggest that HPV may not be present or may not play a prevalent role in endurance exercise performance or blood oxygen saturation at PP_{O_2} s above 95 Torr [18,19,20,25]. In contrast, investigations that have studied HPV in PP_{O_2} s ranging from 70 – 88 Torr have consistently observed improvements in HPV, blood oxygen levels, oxygen consumption, and exercise performance in response to PDE-5 inhibitor administration [5,7,24].

The current investigation also found no effects of acute BRJ consumption on any blood pressure measurements. Like previous investigations, blood pressures were assessed under normoxia, but the current results are in contrast to those observed in many previous studies [11, 12, 26, 27] that observed reductions in systemic blood pressures 2.5 – 3.0 hrs following similar acute BRJ dose strategies. A possible explanation for these discrepancies may be the timing of the blood pressure measurements. Plasma nitrite, cGMP levels, and systemic blood pressure reductions have been shown to peak approximately 150 – 180 min. following acute ingestion of BRJ [26,27]. In the current investigation, resting blood pressure was measured 135 and 155 min post

ingestion. Thus, the early post-ingestion measurement may not have reflected the full response to the treatment. While the latter measurement point was within the peak response window, it occurred five minutes after the completion of the warm-up; thus, changes in blood pressure due to BRJ consumption may have been obscured by those mediated by exercise. Delaying the measurement of pre-exercise blood pressures was initially considered in the study design; however, it was decided to coincide the peak physiological response time window with the exercise portion of the protocol so that possible treatment-mediated changes in physiological and performance variables associated with exercise would be more prevalent. Finally, blood pressures were not measured under hypoxic conditions because previous work [28] and pilot data collected in our laboratory suggest that the small reducing effect of BRJ supplementation on systemic blood pressure would be masked by the relatively large reductions seen in response to hypoxia.

10. CONCLUSION

Red beetroot juice did not affect systemic blood pressure or exercise performance in trained cyclists exercising in hypoxia ($PP_{O_2} = 86$ Torr). Furthermore, exercising oxygen consumption, SpO_2 , and economy were unaffected by acute BRJ supplementation. The lack of response of these physiological variables suggest that acute BRJ supplementation does not alleviate hypoxic pulmonary vasoconstriction or improve exercise performance in this environment.

LIMITATIONS

Although we were unable to directly assess HPV, previous investigations utilizing PDE-5 inhibitors [5,6,7] showed concurrent improvements in HPV and indirect physical markers of HPV such as SaO_2 , oxygen consumption, and performance during exercise in hypoxia. Care should be taken when considering these results.

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