Original Research

Effect of Beetroot Juice on Moderate-Intensity Exercise at a Constant Rating of Perceived Exertion

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ABSTRACT

International Journal of Exercise Science 8(3): 277-286, 2015. Dietary nitrate supplementation has been shown to reduce oxygen consumption at a fixed work rate. We questioned whether a similar effect would be observed during variable work rate exercise at a specific rating of perceived exertion (RPE), as is commonly prescribed for aerobic training sessions. Using a double-blind, placebo controlled, crossover design, ten females (25 ± 3 years; VO_2peak 37.1 ± 5.3 ml/kg/min) performed two 20-min cycle ergometer trials at a constant RPE of 13 (somewhat hard) 2.5 hours following ingestion of 140 ml of concentrated beetroot juice (12.9 mmol nitrate), or nitrate-depleted placebo. Performance was measured in terms of total VO_2 (L) consumed and total mechanical work (kJ) accomplished across each trial. Following each experimental trial, subjects rode at 75W for an additional 5 min to determine the effect of beetroot juice on fixed work rate exercise. Coefficients of variation in total VO_2 (L) and work performed (kJ) during the RPE 13 clamp trials were 8.2 and 9.5%, respectively. Consumption of beetroot juice did not affect total VO_2 or work performed during RPE 13 exercise, but lowered resting systolic blood pressure by ~5 mmHg (P=0.041) and oxygen consumption at 75W by ~4% (P=0.048), relative to placebo. Since the effect of beetroot juice on oxygen consumption is small and may be masked by daily variability during self-regulated exercise, it is unlikely to have a notable effect on daily training.

KEY WORDS: Nitrate, nitrite, nitric oxide, RPE, altitude, reliability

INTRODUCTION

Dietary nitrate (NO_3^-) supplementation, particularly in the form of beetroot juice, has become popular in light of recent studies documenting its ergogenic effects on exercise economy/efficiency and endurance performance [reviewed in (15)] . The effects of nitrate supplementation are believed to be the result of increased nitric oxide (NO) bioavailability following a series of reductions ($NO_3^- \rightarrow NO_2^- \rightarrow NO$) in the entero-salivary and peripheral circulatory systems [reviewed in (21)]. While the vasodilatory effects of NO are well known (14, 33), NO's ability to acutely reduce muscle ATP utilization (2) and mitochondrial oxygen consumption (19) are remarkable because they challenge the tenet that the metabolic cost of steady-state exercise at a fixed work rate is largely invariant {reviewed in (25)]. Such a rapid and fundamental change in exercise metabolism may have an impact on daily training and implications for exercise prescription.

To date, the majority of studies evaluating the ergogenic effects of dietary nitrate have focused on high-intensity, exhaustive efforts, including graded exercise, time-to-exhaustion, and time trial tests [reviewed in (15)]. A recent meta-analysis concluded that dietary nitrate supplementation was associated with small, but potentially meaningful, effects on high-intensity exercise performance (13). While high-intensity efforts may simulate competitive events, daily exercise for training, fitness, or rehabilitation is generally prescribed at much lower intensity. Dietary nitrate supplementation has been shown to reduce the oxygen cost of submaximal exercise at fixed work rates eliciting 45-80% of peak oxygen consumption (VO_2peak) (3, 20); however, it is unknown if a similar effect would be observed if subjects were allowed to self-regulate their work rate to maintain a constant perceived intensity, as would be done during training sessions based on ratings of perceived exertion (RPE).

Exercise at a constant RPE has been proposed as a model to evaluate performance (29), yet few studies have used such "RPE clamp" protocols (24, 30). Recent work from Parfitt and colleagues, showed that 30 min of perceptually-regulated exercise at an intensity perceived as "somewhat hard" [RPE of 13 out of 20 (6)] improved fitness over eight weeks of training (24). Intrigued by these results, we reasoned that an RPE clamp protocol might be used to assess the effects of dietary nitrate supplementation on self-regulated, moderate-intensity exercise performance in two ways. First, by measuring total oxygen consumption (VO_2) across a fixed duration, we could test the hypothesis that dietary nitrate supplementation reduces the oxygen cost of exercise at a prescribed training intensity. Alternatively, if supplementation altered subjects' perception of effort and allowed them to exercise at a higher work rate (23), subjects would perform more mechanical work across the duration of the test.

In this study, we used an RPE clamp model to determine the effects of acute dietary nitrate supplementation - in the form of beetroot juice – on self-regulated exercise performance using a double-blind, placebo-controlled, crossover design.

METHODS

Participants

Based on an a priori power analysis using data from previous beetroot juice studies, a sample size of ten subjects was targeted. Volunteers were recruited from classes at the University of Colorado Colorado Springs (UCCS). From the pool of male and female volunteers, ten females (age 25 ± 3 years; height 165 ± 8 cm; weight 59 ± 11 kg) were randomly selected. All subjects had resided at low altitude in the Colorado Springs metropolitan area (1800 to 2000 m) for at least three months and participated in some form of regular exercise, but none were competitive cyclists. Subjects were familiar with the cycle ergometry, metabolic measurements, and the 6 to 20 RPE scale (6) from previous exercise tests in the laboratory – including incremental exercise tests to maximal exertion. Each subject was informed of the risks and gave their expressed written consent. This project was approved by the UCCS Institutional Review Board (#14-2014) and conducted in accordance with the Declaration of Helsinki.

Protocol

All testing was performed in the UCCS Human Performance Laboratory (1950 m, barometric pressure ~605 mmHg). Ambient temperature was held at 22°C by a thermostat and fan.

Subjects were asked to refrain from high-nitrate foods (e.g. beets, spinach, celery, lettuce), antibacterial mouthwashes and gums know to blunt oral conversion of nitrate to nitrite (12), alcohol, and strenuous exercise for 24 hours. Caffeine intake was restricted for six hours prior to testing. Subjects recorded their food and fluid intake and were asked to replicate their diet prior to the next trial. Subjects ingested two 70-ml bottles of concentrated beetroot juice (12.9 mmol nitrate), or nitrate-depleted placebo (BEET IT Sport Shot, James White Drinks, Ipswich, UK), 150 minutes prior to testing along with a light meal. Subjects were then allowed to consume only water until the start of the experimental trial. No food and fluid intake was allowed during exercise.

Subjects performed one practice and two experimental trials of an RPE clamp protocol to evaluate the effect of dietary nitrate supplementation using a randomized, double-blind, placebo-controlled, counter balanced, crossover design, with a seven day washout between trials. Prior to exercise, subjects rested for ten min in a seated position with their backs, arms, and feet supported to assess resting blood pressure. After adjusting the ergometer (Dynafit Velotron, RacerMate Inc., Seattle, WA) to standardize fit, subjects warmed up for five min at an RPE of 9 (very light), then rode for 20 min at an RPE of 13 (somewhat hard). Subjects were blinded to all external feedback, except for elapsed time, and were instructed to adjust resistance and pedal cadence as needed to maintain the appropriate level of intensity. An RPE chart was placed one meter in front of the ergometer for visual reference. Following the RPE 13 clamp protocol, cool down exercise was performed for five min at 75 Watts to evaluate the effect of dietary nitrate on fixed work rate exercise.

Subjects' resting systolic, diastolic and mean blood pressures were measured using an automated sphygmomanometer (CBM-7000, Colin Medical Instruments, San Antonio, TX). Resting heart rate and arterial oxygen saturation were assessed by finger pulse oximetry (Nellcor N-200, Covidien, Mansfield, MA). The average of two measurements obtained during the last five min of rest was used for statistical analysis.

During exercise, metabolic measurements were assessed by indirect, open-circuit calorimetry (Parvomedics TrueMax 2400, Salt Lake City, UT) with subjects breathing through a two-way, non-rebreathing valve and mouthpiece. Across the 20-min periods at RPE 13, total VO_2 (L) was calculated by summing 60 s averages of VO_2 (L/min). Total work (kJ) was calculated as the product of average power (W) and test duration (1200 s). For fixed work rate exercise at 75 W, VO_2 (L/min) was averaged over the last two min. Heart rate was integrated into the metabolic cart using a chest strap and telemetric receiver (Polar Electro, Lake Success, NY).

Statistical Analysis
To evaluate the reliability of the RPE 13 clamp protocol, coefficients of variation (CV) and Cronbach's alpha statistics for total VO_2 and mechanical work accomplished during the protocol were calculated from a retrospective analysis of matched subjects who preformed three repeated trials on separate days without dietary nitrate supplementation (UCCS IRB #15-030). To evaluate the effect of dietary nitrate supplementation on the RPE 13 clamp protocol, paired t-tests were used to test the hypotheses that beetroot juice would reduce total VO_2 (L), or alternatively, increase total mechanical work accomplished, relative to placebo. Similarly, a paired t-test was used to test that hypothesis that nitrate supplementation would reduce the rate of VO_2 (L/min) during exercise at a fixed work rate of 75 W. Significance was inferred at $P < 0.05$. Data are presented as mean ± standard deviation (SD).

RESULTS

All ten subjects completed the beetroot juice trials. Due to a computer malfunction, one subject's data for a single trial was corrupted. Data for the remaining nine subjects (age 24 ± 3 years; height 166 ± 8 cm; weight 60 ± 11 kg; VO_2peak 36.1 ± 4.7 ml/kg/min) were analyzed. Beetroot juice lowered resting systolic blood pressure relative to placebo prior to exercise (107 ± 13 vs. 112 ± 14 mmHg, P =0.041; Figure 1), but did not affect resting diastolic (62 ± 8 vs. 64 ± 9 mmHg, P = 0.265), or mean arterial pressures (77 ± 9 vs. 80 ± 10 mmHg, P = 0.147). There were no differences in resting HR (65 ± 14 vs. 69 ± 7 bpm, P = 0.799) or SpO_2 (98 ± 1 vs. 98 ± 1%, P = 0.755) following beetroot juice consumption, relative to placebo.

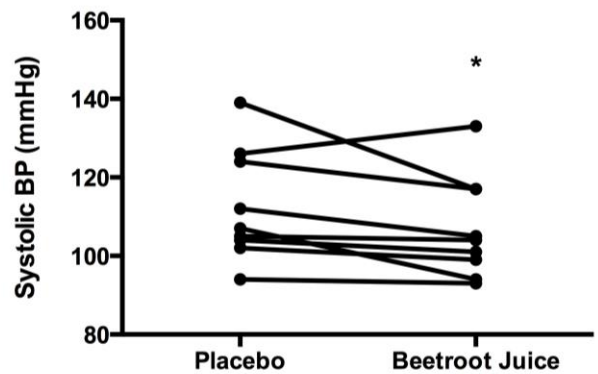

Figure 1. Individual responses in resting systolic blood pressure 2.5 hours post ingestion of 140ml beetroot juice (12.9 mmol nitrate) or nitrate-free placebo in young, healthy females (n=9). * Beetroot juice reduced average systolic blood pressure by ~5 mmHg (P = 0.041).

Table 1. Intrasubject coefficients of variation across RPE 13 clamp trials (n=8).

Variable	Trials			
	All	1 & 2	1 & 3	2 & 3
Total VO2 (L)	8.2 ± 3.5	7.1 ± 4.6	9.0 ± 6.0	6.8 ± 2.3
Total Mechanical Work (kJ)	9.5 ± 3.7	8.8 ± 4.7	10.7 ± 6.6	6.9 ± 2.8

Values are mean % ± SD. Volume of oxygen consumption (VO2).

The CVs for total VO_2 and mechanical work accomplished across repeated RPE 13 clamp trials were 8.2 ± 3.5% and 9.5 ± 3.7%, respectively (Table 1). Cronbach's alpha reliability coefficients for total VO_2 and mechanical work accomplished were 0.90 and 0.88, respectively. Beetroot juice did not decrease total VO_2 (P = 0.352) or increase mechanical work (P = 0.346) accomplished, relative to placebo (Table 2, Figure 2). However, the rate of VO_2 at a fixed work rate of 75 W was ~4% lower ($P = 0.048$) following ingestion of beetroot juice compared to placebo (Table 3, Figure 3).

Table 2. Effect of beetroot juice on 20 min of exercise at RPE 13 (n=9).

Variables	Placebo	Beetroot Juice
Metabolic		
Total VO2 (L)	30.31 ± 4.65	30.01 ± 4.10
VO2 (L/min)	1.51 ± 0.23	1.50 ± 0.20
% VO2peak	70 ± 12	70 ± 13
VCO2 (L/min)	1.47 ± 0.24	1.46 ± 0.19
RER	0.97 ± 0.04	0.97 ± 0.04
VE (L/min)	50.8 ± 8.0	50.4 ± 6.4
Heart rate (bpm)	149 ± 17	152 ± 14
Mechanical		
Total Work (kJ)	29.8 ± 6.1	30.3 ± 5.3
Mean Power (W)	104 ± 21	106 ± 18
% Peak Power	52 ± 13	54 ± 11

Values are mean ± SD. Volume of oxygen consumption (VO2), carbon dioxide production (VCO2), respiratory exchange ratio (RER), and ventilation expired (VE).

DISCUSSION

There were three main findings from this study: 1) Total VO_2 (L) and work accomplished (kJ) over 20 min of cycling at a constant RPE of 13 provided reliable assessments of self-regulated, submaximal exercise performance. 2) While a single dose of beetroot juice (12.9 mmol nitrate) reduced resting systolic blood pressure and VO_2 (L/min) at a fixed work rate (75 W), 3) it did not improve performance measured during the RPE 13 clamp protocol. Because the effects of beetroot juice were small, they may have been masked by daily variation in the metabolic response to self-regulated exercise. It is thus unlikely that acute consumption of beetroot juice has observable effects on daily exercise training.

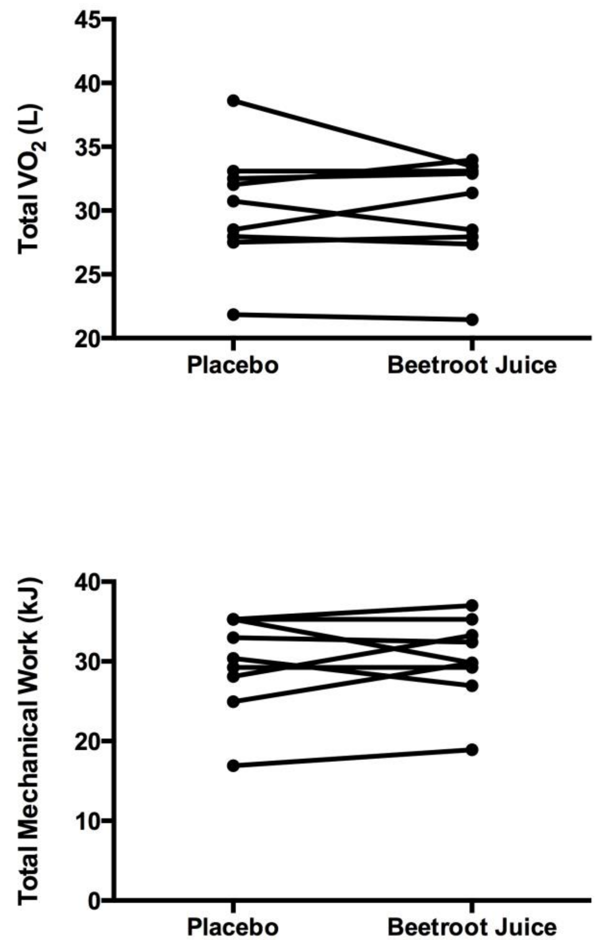

Figure 2. Total VO_2 consumed and mechanical work performed over 20 min of exercise at rating of perceived exertion (RPE) of 13 (somewhat hard). Data were collected from young, healthy females (n=9) 2.5 hours post ingestion of 140ml beetroot juice (12.9 mmol nitrate) or nitrate-free placebo in. Beetroot juice did not affect total VO_2 ($P = 0.352$).

An ideal performance test must be valid and reliable. Because training for a fixed duration at a specific RPE is a common paradigm for exercise prescription (1) and is effective at improving fitness (24), the RPE 13 clamp protocol has inherent face validity. The calculated CVs indicate that

Table 3. Effect of beetroot juice on exercise at 75 W (n=9).

Variables	Placebo	Beetroot Juice
VO2 (L/min)	1.30 ± 0.11	1.25 ± 0.08*
% VO2peak	61 ± 11	58 ± 11*
VCO2 (L/min)	1.21 ± 0.12	1.13 ± 0.07
RER	0.93 ± 0.05	0.90 ± 0.03
VE (L/min)	44.6 ± 5.2	41.0 ± 3.4
Heart rate (bpm)	149 ± 18	147 ± 18

Values are mean ± SD. * Different from placebo ($P < 0.05$). Volume of oxygen consumption (VO2), carbon dioxide production (VCO2), respiratory exchange ratio (RER), and ventilation expired (VE).

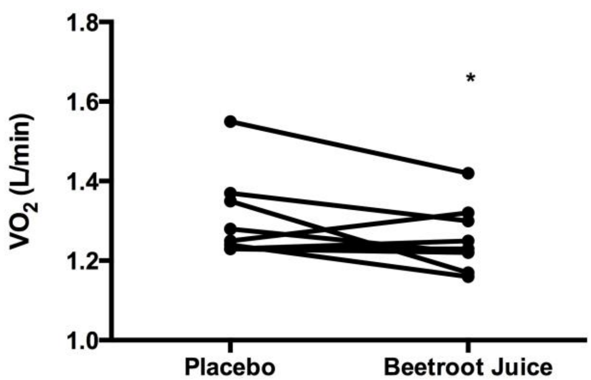

Figure 3. Individual responses in VO_2 (L/min) during exercise at 75W 2.5 hours post ingestion of 140ml beetroot juice (12.9 mmol nitrate) or nitrate-free placebo in young, healthy females (n=9). * Beetroot juice reduced average VO_2 (L/min) by ~4% ($P = 0.048$).

metabolic and mechanical data obtained over 20 min of cycling at an RPE of 13 varies by < 10% from day to day. By comparison, CVs associated with the RPE clamp protocol were not as good as values typically reported for time trial efforts (< 5%), but fell within the range of values reported for time to exhaustion tests (< 26%), two commonly accepted laboratory measures of maximal cycling performance that have been positively affected by beetroot juice supplementation (3, 7, 8, 18, 28). Additionally, Cronbach's alpha coefficients for the RPE clamp performance measures ranged from 0.88 to 0.90, indicating a strong correlation across repeated trials and therefore high degree of reliability (9).

While the reliability of RPE during progressive intensity tests has been questioned (17), our favorable results are in accordance with previous research on steady-state exercise at an RPE of 13. Eston and Williams (10) also reported a high degree of reliability in VO_2 measured after four min at RPE 13 across three trials. Interestingly, they noted improvement in the strength of the correlation as subjects habituated to the protocol. We observed a similar effect in both performance measures, as the CVs between the second and third trials dropped below 7% (Table 1). Overall, since RPE 13 clamp protocol has a high level of face validity and comparable reliability to other accepted performance tests, total VO_2 and mechanical work accomplished during the protocol appear to be suitable measurements to assess performance during submaximal, self-regulated efforts.

Contrary to our hypothesis, acute consumption of beetroot juice did not reduce total VO_2 or increase work accomplished during the RPE 13 clamp protocol. It is possible that the single dose of beetroot juice was insufficient to elicit changes in these measures and that chronic supplementation (>5 days) may have been more effective (31). Yet, there are several studies that have shown positive effects from single doses of beetroot juice, comparable to those used in our study (4, 18, 23, 34). Specifically, we selected a dose (12.9 mmol nitrate) based on evidence that acute consumption of 8 to 16 mmol nitrate reduced VO_2 during submaximal exercise by 2-3% and increased time to exhaustion (35). Empirically, we are confident that a sufficient dose was given and maintained throughout the protocol because we saw evidence of efficacy before and after the RPE clamp protocol. Before, we observed reductions in resting systolic blood pressure (~5 mmHg) prior to exercise that were commensurate with previous studies [reviewed in (27)]. After, VO_2 was reduced by ~4% during steady-state exercise immediately after the RPE 13 clamp protocol, which was again consistent with expected effects from nitrate supplementation (3, 20). These findings gave us confidence that the dose provided was sufficient during the experimental protocol.

We believe that the small effect beetroot juice has on VO_2 may have been masked by the ~8% daily variation in total VO_2 across the trial discussed above, hence resulting in non-significant effects. Additionally, because subjects varied their work rate to maintain an RPE of 13, periods of steady-state VO_2 were not observed. Respiratory exchange ratios for each individual fluctuated throughout the protocol, with the majority of subjects (seven of nine) registering values over 1.00 for at least two min during the protocol. This variation may have reduced our statistical power and thus our ability to detect significance (see Limitations). Also, while dietary nitrate supplementation has been shown to hasten oxygen kinetics during transitions from moderate- to severe-intensity exercise, and thereby prolong time to exhaustion, improvements in oxygen kinetics have not been seen at lower intensities (7). It is thus possible that the continual, although slight, adjustments in work rate necessary to maintain moderate-intensity exercise precluded our ability to detect effects of dietary nitrate supplementation on total oxygen consumption and mechanical work accomplished. Future work at a higher RPE, where dietary nitrate may improve oxygen kinetics during transitions in work rate, may therefore be warranted.

Subjects were recruited from classes that were predominantly (>70%) female. This sampling bias resulted in a female-only sample. To the best of our knowledge, only one other study has been performed on an exclusively female population (5) and reported similar reductions in systolic blood pressure and VO_2 at fixed submaximal work rates to those reported in this study and others with both male and female subjects. Based on the literature to date, there does not seem to be a differential response to beetroot juice between sexes, but this remains to be rigorously tested.

Our subjects were recreationally active females with VO_2peak values of ~35 ml/kg/min assessed at an elevation of 1950 m. To keep these values in perspective, it is important to note that at this altitude, VO_2peak values underestimate sea level values by ~10% (11). Previous studies have suggested that dietary nitrate supplementation may be more effective at altitude where reduction of nitrite to NO may facilitate matching of O_2 availability to demand (16, 22, 32). However, whether the added effects of dietary nitrate supplementation in acute hypoxia are sustained after acclimatization to altitude – as in our subjects – remains to be determined.

Although the sample size was sufficient to detect the effects of nitrate supplementation on resting blood pressure and VO_2 at a fixed work rate, it may have been insufficient to detect effects during the RPE 13 clamp protocol. Post hoc power analyses using calculated effect sizes for total VO_2 and mechanical work accomplished (0.13 and 0.14, respectively) revealed that a sample size of at least 250 subjects would have been necessary to maintain a 70% chance of detecting a truly significant result. These results support our argument that effects of beetroot juice were small and likely masked by daily variation self-regulated exercise.

The popularity of dietary nitrate supplementation has risen dramatically over the last few years. Although the main ergogenic effects have been seen in tests of exhaustive exercise capacity, reports of improved submaximal efficiency/economy imply that positive effects may also be seen during daily training sessions of moderate intensity. The RPE 13 clamp protocol was used to mimic a basic aerobic training session. Because we found no effect of beetroot juice on this form of self-regulated, submaximal exercise it is unlikely acute dietary nitrate supplementation will affect the volume or quality of daily training. This argument is supported by a recent study showing that daily consumption of beetroot juice while training in hypoxia (equivalent to 4000 m) did not improve sea level VO_2max or 30-min time trial performance over six weeks relative to a placebo (26). We thus do not recommend the daily consumption of beetroot juice to increase performance during moderate-intensity aerobic training sessions.

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