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Original Research

Effects of Single-Dose Dietary Nitrate on Oxygen Consumption During and After Maximal and Submaximal Exercise in Healthy Humans: A Pilot Study

GENEVIEVE M. KOCOLOSKI*, and ANNE R. CRECELIUS‡

Department of Health and Sport Science, University of Dayton, Dayton, OH, USA

*Denotes undergraduate student author, ‡Denotes professional author

ABSTRACT

International Journal of Exercise Science 11(3): 214-225, 2018. Dietary nitrate (NO_3^-) has been shown to reduce oxygen consumption (VO_2) during moderate to high-intensity (e.g. time to fatigue, time trials) exercise and often in trained athletes. However, less is known regarding prolonged exercise and the potential impact of NO_3^- on post-exercise excess oxygen consumption (EPOC), particularly in untrained individuals, who may have different metabolic goals during exercise than trained individuals. We tested the hypothesis that acute nitrate supplementation in the form of beet root juice will significantly decrease both VO_2 during maximal exercise and EPOC in both maximal and submaximal exercise trials. Eight young, moderately active, healthy males (age: 24.8±1.4 years, body mass index: 23.7±0.4 kg/m^2; VO_2max: 34.2±3.9 ml/kg/min) performed step-wise maximal cycle exercise (n=4) and prolonged submaximal cycle exercise (n=6) (45 min; 38±2% of max work rate) in control (anti-bacterial mouthwash) and acute NO_3^- supplemented conditions [70ml concentrated beet root juice (0.4g NO_3^-), 2 hrs prior to exercise] on separate occasions. Measurements of VO_2 (indirect calorimetry), arterial blood pressure (MAP; sphygmomanometry), and heart rate (HR; ECG) were made before, during, and following exercise bouts. NO_3^- reduced MAP at rest ~1-3mmHg. However, NO_3^- had no impact on VO_2 during maximal (VO_2max, Ctrl: 34.2±3.9 ml/kg/min vs NO_3^-: 31.7±4.4 ml/kg/min), submaximal exercise (average of min 25-45, Ctrl: 24.6±2.4 ml/kg/min vs NO_3^-: 26.8±3.3 ml/kg/min) or EPOC (area under the curve, Ctrl: 0.57±0.24 L vs NO_3^-: 0.66±0.16 L). Thus, while NO_3^- supplementation may have performance benefits in elite athletes exercising at high intensities, in recreationally active males, there appears to be little impact on changes in VO_2 due to maximal or submaximal prolonged exercise.

KEY WORDS: Cycle ergometry, nitric oxide, metabolism, supplement

INTRODUCTION

Aerobic exercise is an important factor in combating the health issues brought on by the widespread presence of cardiovascular disorders (14). Aerobic exercise has been known to improve cardiovascular structure and function while increasing energy expenditure and providing psychological benefit (11). As the substrate for aerobic metabolism, oxygen is essential during bouts of exercise during which the body's muscles are contracting with more power. During high-intensity exercise, 90% of the body's energy is spent in the muscles (17). As the workload of an exercise bout increases in terms of power output, the demand for oxygen increases accordingly (10). Additionally, with an increase in oxygen consumption also comes an increase in energy expenditure as they are directly and linearly related.

At the beginning of exercise, the body's demand for oxygen increases faster than its rate of consumption (15). This difference between the oxygen demand and consumption creates an "oxygen deficit" at the beginning of exercise, in which the amount needed is not available. At the cessation of exercise, the body maintains an elevated rate of oxygen consumption in order to compensate for the prior deficit. This prolonged elevated rate of oxygen consumption is referred to as excess post-exercise oxygen consumption, or EPOC (13).

Multiple variables have been shown to impact EPOC, including duration and intensity (25, 1), menstrual status (12), nutritional status (2, 6, 24), and exercise mode (5). Additionally, given that cardiac output is a primary determinant of oxygen consumption, or VO_2, (along with the arterial-venous oxygen content difference), factors that impact cardiac output may lead to alterations in VO_2, and further EPOC, if not reflexively compensated for. At the level of the muscle, any substance capable of modulating mitochondrial respiration will impact VO_2 as well. One such substance that is capable of both hemodynamic and metabolic effects is the gaseous signaling molecule nitric oxide. Nitric oxide can be synthesized within the body via nitric oxide synthase during the conversion of L-arginine to L-citrulline. Additionally, the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) to nitric oxide (NO) can also increase NO bioavailability (21).

NO derived from ingested organic NO_3^- has been shown to have significant effects hemodynamically. NO is the substance responsible for relaxation of blood vessels, which will in turn increase oxygen delivery to the rest of the body. Research indicates that it plays a key role in an increase in blood flow during exercise (27). In addition to its hemodynamic effects, it has also been shown to stimulate mitochondrial biogenesis (9, 22), or production of new mitochondria. One supplement in particular that has been of recent interest is NO_3^- in the form of beetroot juice. This supplement has been shown to decrease VO_2 at a given workload (18), increase performance in time trial time (7), and decrease diastolic blood pressure (20). Additionally, beetroot juice reduces VO_2 without altering mitochondrial efficiency (28) and has been shown to have little effect in cases of submaximal exercise (4). However, there is a lack of published data regarding the effect of dietary nitrate supplementation on EPOC as well as during exercise in untrained individuals.

Due to the lack of published data, a pilot experiment was designed to determine the effects. It was hypothesized that acute nitrate supplementation in the form of beet root juice will significantly decrease VO_2 during maximal exercise and also decrease excess post-exercise oxygen consumption (EPOC) in both maximal and submaximal exercise trials.

METHODS

Participants

Subjects were recruited from the University of Dayton and the surrounding community to take part in this study. An initial screening of 8 healthy, male individuals aged 19-31 took place prior to the testing sessions. These screenings ensured that each subject was a non-smoker, non-obese man with a BMI of less than 30 kg/m^2, normotensive (<140/90 mmHg), moderately active, and not taking any medications. Body composition was determined during the initial screening visits using air displacement (Bod Pod, COSMED, Chicago, IL). Subject characteristics are shown in Table 1. N=4 for the maximal exercise tests and n=6 for the submaximal exercise tests.

Table 1. Subject characteristics for the total subject population (n=8).

Age	24.88±1.41
Height (cm)	181.29±2.88
Weight (kg)	78.15±3.17
Body Fat %	15.81±1.50
BMI (kg/m^2)	23.71±0.40

Protocol

Maximal testing consisted of two graded maximal exercise tests, one in control and one in the condition. A sample protocol is shown in Figure 1.

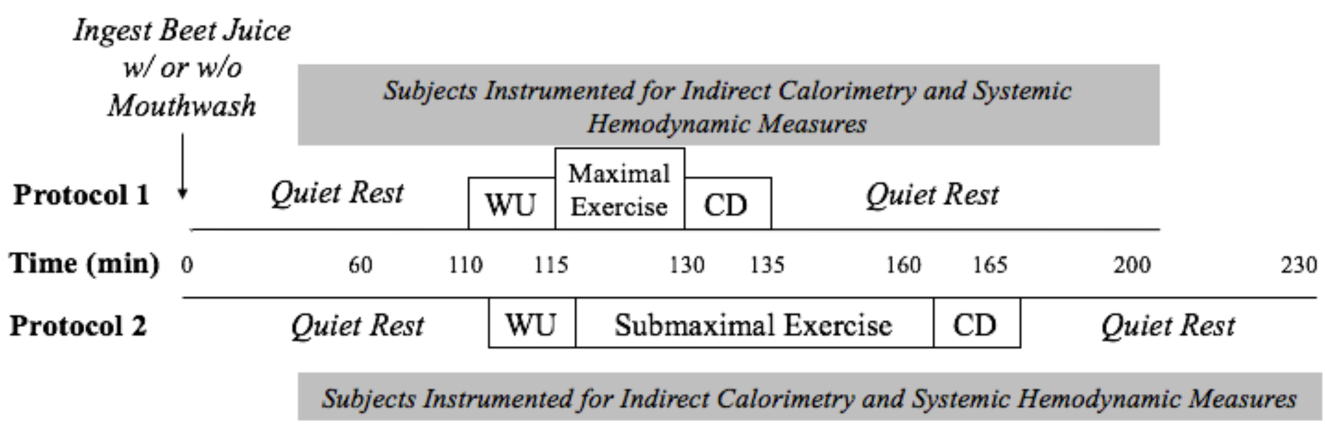

Figure 1. Protocol of both maximal and submaximal exercise tests. WU=warm up; CD=cool down; n (Protocol 1) = 4; n (Protocol 2) = 6.

Immediately upon arrival to the lab on a testing day, subjects rinsed their mouth with three separate 70ml amounts of either mouthwash (control) or water (condition). They then ingested 70ml of Beet It Sport Shot (Beet-It USA, Greenwood Village, CO). After ingesting the beetroot juice, subjects rested in a comfortable position without instrumentation. After 60 minutes, subjects were instrumented with a mask to measure indirect calorimetry, a heart rate monitor, and a blood pressure cuff. The subjects then rested supine on a bed for 45 minutes before transferring to the cycle ergometer. After transfer (5 minutes), each subject completed a five-minute warm-up followed by a graded maximal exercise test to determine VO_2max (7-10 min). During each test, subjects maintained a speed of 70 rpm on the bicycle with the predetermined workload increasing by 0.5 kp every minute. Exercise ceased when 1) respiratory exchange ratio (RER) rose above 1.2, 2) RER stopped increasing with progressive workloads, or 3) the subject could no longer maintain the cadence. The point at which the subject's oxygen consumption rose the highest during maximal exertion was determined as VO_2max. Following the test, each subject completed a five-minute cool-down and was given five minutes to rest without the mask. Subjects then transitioned back to the bed and remained supine for the next 60 minutes to determine EPOC. Resting, post-exercise measures were taken to determine VO_2, heart rate, and blood pressure.

Some subjects (n=2) who took part in Protocol 1 also participated in Protocol 2, as well as an additional 4 subjects for a total of 6 males in Protocol 2. The submaximal testing consisted of two experimental visits: 45 minutes at a given percentage of their control maximum workload in control and 45 minutes at a given percentage of their control maximum workload in the nitrate condition. A sample submaximal protocol is shown in Figure 1.

Immediately upon arrival to the lab on a testing day, subjects rinsed with three separate 70ml amounts of either mouthwash (control) or water (condition). They then ingested 70ml of Beet It Sport Shot. After ingesting the beetroot juice, subjects rested in a comfortable position without instrumentation. After 60 minutes, subjects were instrumented with a mask to measure indirect calorimetry, a heart rate monitor, and a blood pressure cuff. The subjects then rested supine on a bed for 45 minutes before transferring to the cycle ergometer. After transfer (5 minutes), each subject completed a five-minute warm-up and then 45 minutes of submaximal exercise at a given workload determined by a certain percentage of their VO_2max in a given condition. Following the test, each subject completed a five-minute cool-down and was given five minutes to rest without the mask. Subjects then transitioned back to the bed and remained supine for the next 60 minutes to determine EPOC. Resting, post-exercise measures were taken to determine VO_2, heart rate, and blood pressure.

Indirect calorimetry was performed with a Parvo Metabolic Cart (Parvo Medics, Sandy, UT) after standard manufacturer calibration. These data yielded oxygen consumption and energy expenditure. The coefficient of variation of repeated sampling for oxygen consumption within the laboratory was 3.9%. Blood pressure was determined using the Datascope Passport 2 Patient Monitor (Datascope, Chicago, IL) during rest and manually using a stethoscope and sphygmomanometer during exercise (by same researcher). Heart rate was measured using a Polar H7 Bluetooth Smart Chest Transmitter (Polar Electro, Lake Success, NY).

Statistical Analysis

VO_2 max, energy expenditure, and workload values were compared using paired t-tests to determine statistical significance, and the p value was set at .05. Standard error was also calculated for each average.

RESULTS

Maximal Testing: Four subjects' data were included in analysis (n=4). Both pre- and post-exercise resting mean arterial pressures were reduced 1-2 mm Hg in the nitrate condition. There was no difference in maximum workload between conditions, shown in Table 2.

Pre-exercise energy expenditure showed no significant change in average energy expenditure between the nitrate condition and control, represented in Table 3 (control: 53.3±13.5 kcal; nitrate:

48.3±13.6 kcal, p=0.80). Average VO_2max of subjects (n=4) in the control condition was 34.2±3.9 ml/kg/min and 31.7±4.4 ml/kg/min in the nitrate condition (p=0.67), as shown in Figure 2.

Table 2. Maximum workloads reached by each subject during maximal trials in both control and nitrate conditions.

Subject	Control	Nitrate
4	3.5 kp	3.5 kp
8	4.0 kp	4.0 kp
9	4.5 kp	4.5 kp
11	4.0 kp	4.0 kp

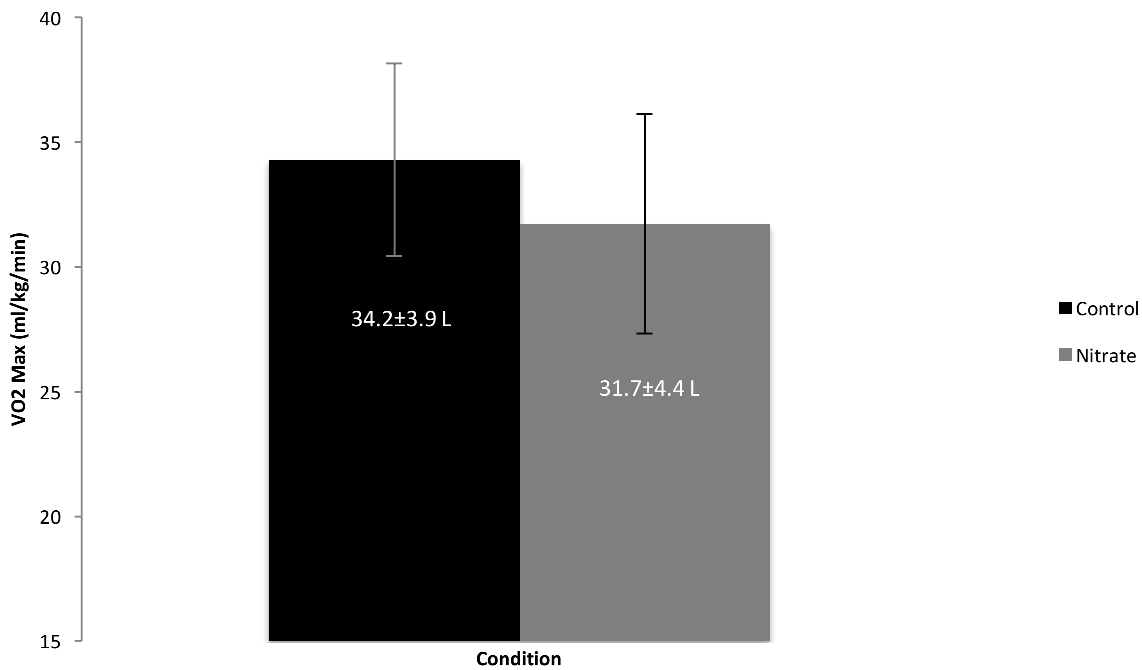

Figure 2. Average VO_2max. Average VO_2max of subjects in control and nitrate conditions.

As shown in table 3, there was no significant change in average energy expenditure during exercise (control: 51.6±8.6 kcal; nitrate: 52.0±9.2 kcal, p=0.97) or in average post-exercise energy expenditure between the nitrate condition and control (control: 136.5±14.4 kcal; nitrate: 113.3±18.2 kcal; p=0.36).

Table 3. Maximal exercise tests- energy expenditure pre-, during and post-maximal exercise.

	PRE-EXERCISE		DURING EXERCISE		POST-EXERCISE	
Subject	Control EE (kcal)	Nitrate EE (kcal)	Control EE (kcal)	Nitrate EE (kcal)	Control EE (kcal)	Nitrate EE (kcal)
4	62.8	64.0	47.0	50.9	137.6	135.0
8	13.1	16.7	31.9	26.6	95.5	67.4
9	66.2	35.5	73.2	62.3	158.8	102.1
11	71.3	77.1	54.1	68.1	154.1	148.7
Mean ± SEM	**53.3 ± 13.5**	**48.3 ± 13.6**	**51.6 ± 8.6**	**52.0 ± 9.2**	**136.5 ± 14.4**	**113.3 ± 18.2**

EE=energy expenditure; kcal=kilocalories, SEM=standard error of measurement.

A curve showing dynamic oxygen consumption throughout pre-exercise resting, maximal testing, and post-exercise resting is shown in Figure 3. The measures of VO_2max are represented here as well, and the elevated rate of oxygen consumption (EPOC) during the control and nitrate conditions throughout the entirety of the post-exercise resting period.

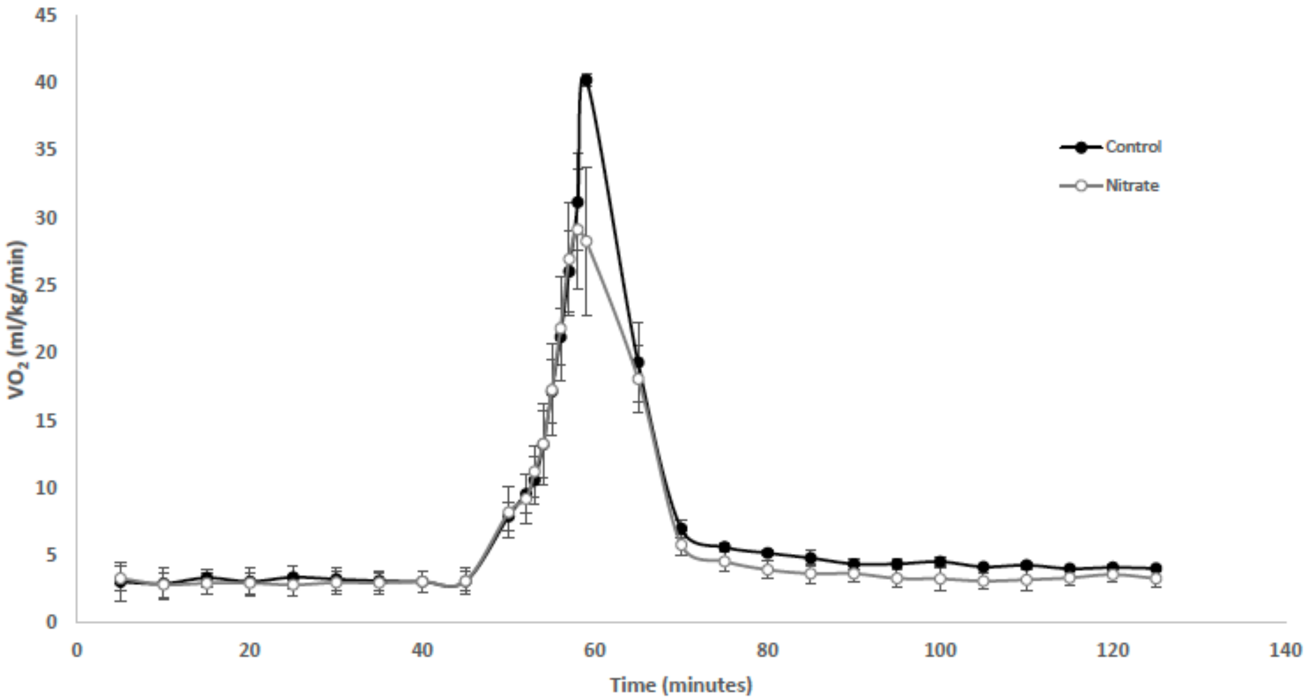

Figure 3. Maximal exercise dynamic oxygen consumption. Average dynamic oxygen consumption throughout the entirety of the maximal exercise testing bouts.

Submaximal Testing: Six subjects' data were included in analysis (n=6). Both pre- and post-exercise resting mean arterial pressures were reduced 1-3 mm Hg in the nitrate condition. No significant change was seen in average total pre-exercise energy expenditure between the nitrate condition and control condition as shown in Table 4 (control: 71.3.43±6.7 kcal; nitrate: 72.1±11.2 kcal; p=.95). Also shown in Table 4, no change was seen in energy expenditure during prolonged submaximal exercise (control: 435.2±75.0, nitrate: 447.4±70.8 kcal, p=0.91). During exercise, no significant change in VO_2 was seen between the nitrate condition and control, shown in Figure 4.

No change was seen in average total post-exercise energy expenditure between the nitrate condition and control as shown in Table 4 (control: 116.1±12.8 kcal, nitrate: 119.3±15.5 kcal; p=0.88).

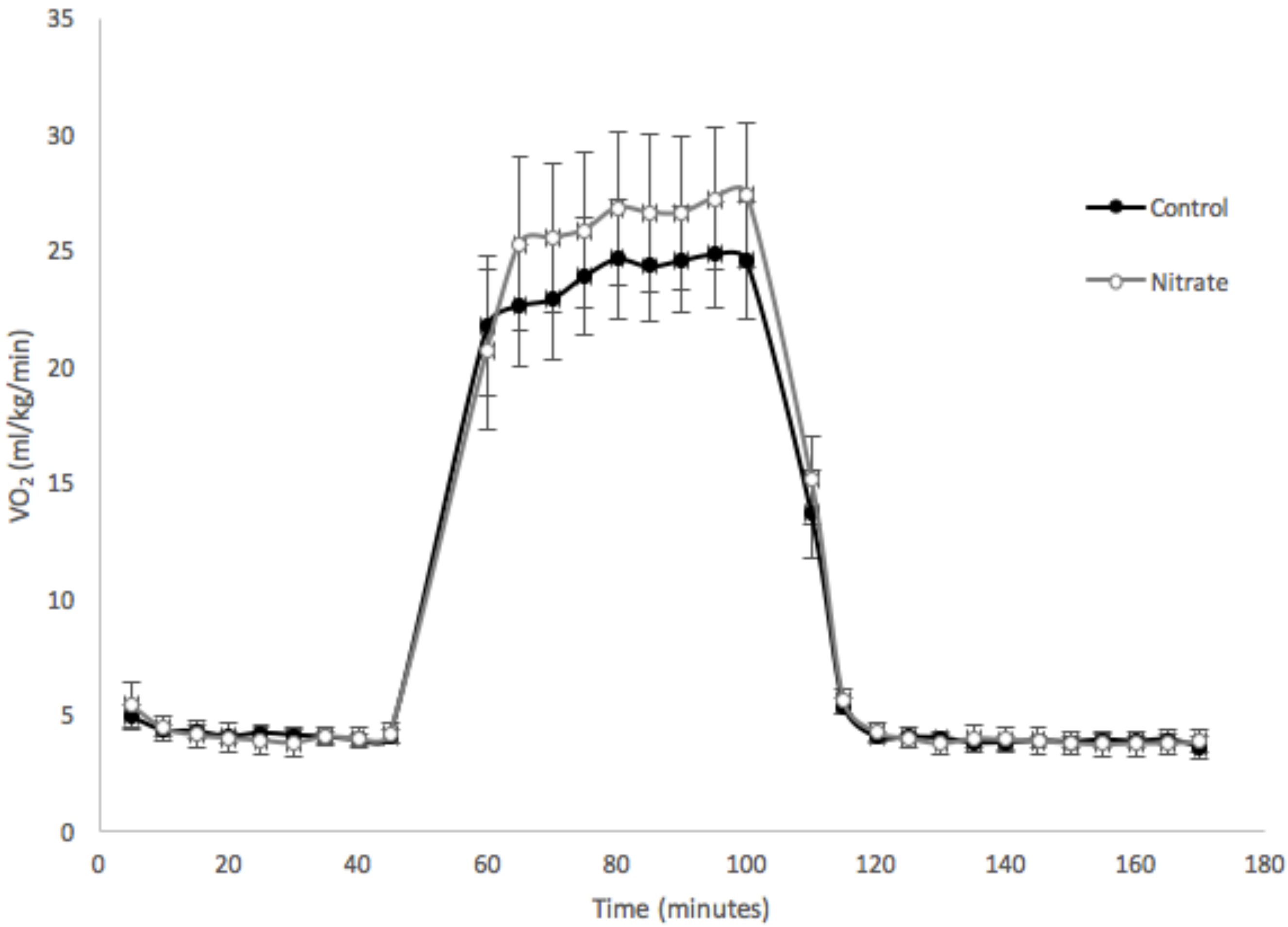

Figure 4. Submaximal exercise dynamic oxygen consumption. Average dynamic oxygen consumption throughout the entirety of the submaximal exercise testing bouts.

Table 4. Submaximal exercise tests- energy expenditure pre-, during and post-submaximal exercise.

	PRE-EXERCISE		DURING EXERCISE		POST-EXERCISE	
Subject	Control EE (kcal)	Nitrate EE (kcal)	Control EE (kcal)	Nitrate EE (kcal)	Control EE (kcal)	Nitrate EE (kcal)
1	93.0	99.3	781.9	677.8	163.0	163.4
2	83.6	92.5	372.1	207.3	139.9	146.2
3	79.3	87.7	433.0	593.6	117.9	134.2
4	61.7	65.0	363.5	403.8	103.0	107.2
7	59.2	63.3	424.2	478.3	97.8	108.8
8	50.7	25.0	236.7	323.5	75.3	55.9
Mean ± SEM	**71.3 ± 6.7**	**72.1 ± 11.2**	**435.2 ± 75.0**	**447.4 ± 70.8**	**116.1 ± 12.8**	**119.3 ± 15.5**

EE=energy expenditure; kcal=kilocalories, SEM=standard error of measurement.

As represented in Figure 5, EPOC was 0.57±0.24 L in the control condition and 0.66±0.16 L in the nitrate condition at the same workload ($p=0.58$). No data from the submaximal trials was significant.

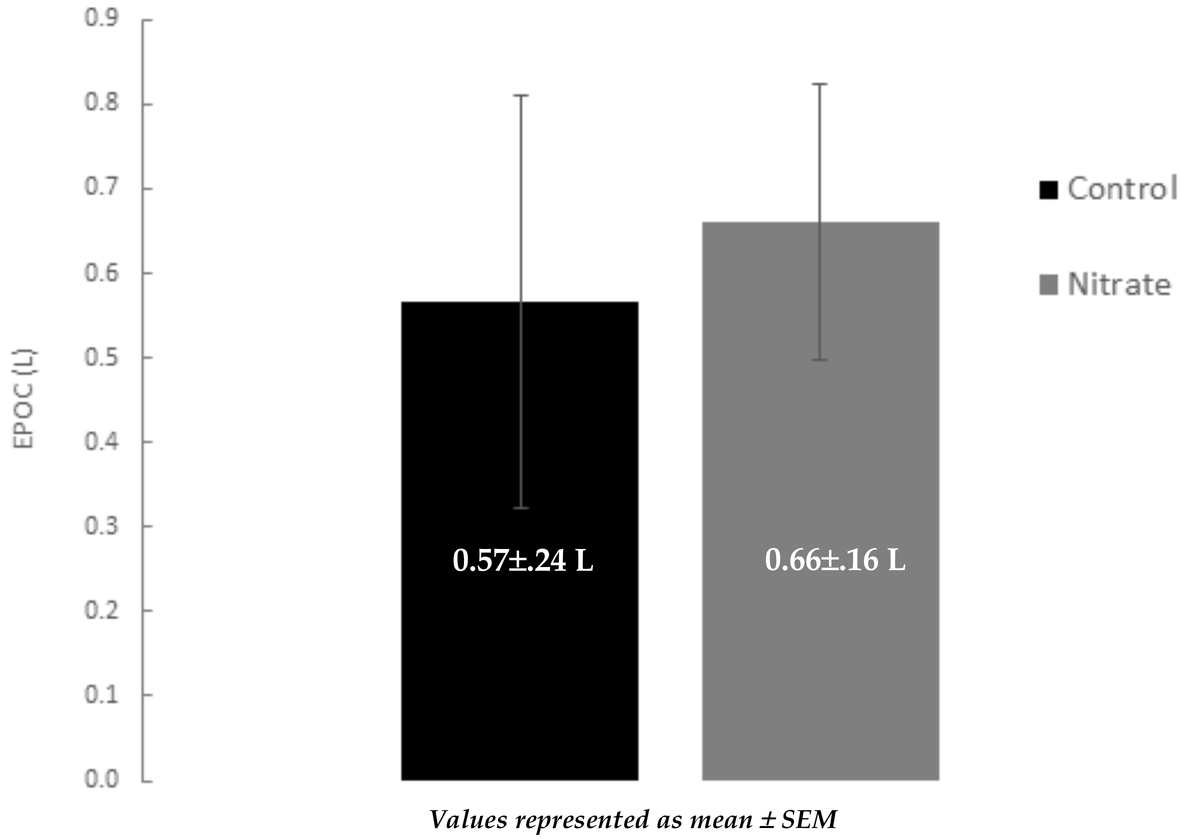

Figure 5. EPOC in nitrate and control conditions. EPOC measurements for 60 minutes following submaximal exercise in both control and nitrate conditions.

DISCUSSION

In this study, VO_2 was measured in both nitrate and control conditions for submaximal and maximal bouts of exercise. Pre- and post-exercise values were measured in addition to those taken during the exercise.

Maximal Exercise: VO_2max has been shown to be attenuated following supplementation with beetroot juice (18). A lower VO_2max while at the same workload would indicate an improvement in exercise economy for that exercise bout when supplementing with beetroot juice. In our study, average VO_2max was unchanged in the nitrate condition when compared with control. Additionally, each subject reached the same workload during the maximal exercise tests in each condition. These data are inconclusive, and unable to support the findings that nitrate supplements in the form of beetroot juice lower oxygen consumption. EPOC was also unchanged in the nitrate condition. Had a lower rate of oxygen consumption been attained during exercise in the nitrate condition creating a smaller oxygen deficit, a lower rate would also be seen during post-exercise measurements, resulting in a lower total EPOC.

Submaximal Exercise: It has been shown that supplementation with beetroot juice may reduce the O_2 cost in moderate-intensity exercise while greatly attenuating VO_2 in maximal trials (3). In more recent studies, it has been shown that while beetroot juice lowers VO_2 during maximal exercise, there is no clear effect of nitrate supplementation on submaximal or endurance exercise (4, 8). In the current study, the submaximal trials showed no difference between conditions; VO_2 was unchanged in nitrate condition as compared to the control condition. Energy expenditure was also unchanged in the nitrate condition. These results showed no significant difference, supporting the finding that there is no real effect during submaximal exercise. Due to an unchanged VO_2 during submaximal exercise in the nitrate condition, EPOC was also unchanged in this condition.

Supplementation Application: When considering whether supplementation is a desirable strategy, the overall goals must be taken into consideration. In the case of a highly fit athlete, lower VO_2 with supplementation (as we observed during maximal exercise) may be beneficial, as it can suggest greater metabolic economy. However, for an untrained person, as were our subjects, often improved economy is not desirable. For example, if someone is engaging in exercise as a means of creating an energy deficit to promote weight loss, decreased VO_2 and therefore energy expenditure would be disadvantageous. Exercise programs for weight-loss often consist of prolonged submaximal exercise and here, unlike some other previous studies (18), we do not show a diminished VO_2 response, nor a diminished EPOC, with nitrate supplementation. Thus, it appears that the present data do not argue against supplementation if weight loss, rather than elite performance is the goal. Additionally, the population used in this study was composed of sedentary-to-moderately active individuals. To date, various studies have shown little to no effect of beetroot supplementation in populations of well-trained athletes (8, 29, 23). Thus, the particular population as well as the goals should also be taken into account when considering supplementation.

Experimental Considerations: One factor to consider in the current study is supplementation efficacy and the lack of plasma nitrate values due to the technical difficulty of these types of measures. We did not collect blood samples, but believed the supplementation to be effective due to changes reflected in a decrease in blood pressure (Δ1-3 mm Hg) in pre- and post-exercise rest periods. Additionally, previously this same dose of concentrated beet root juice has been shown to significantly increase plasma nitrate levels (26). This study used a mouthwash rinse as the control instead of nitrate-depleted beetroot juice. This method has been shown to inhibit the physiological effects of nitrite, and further nitric oxide, derived from the oral reduction of nitrates (19). Additionally, while a larger dose of beetroot juice may result in a larger ergogenic effect (16), the amount used in this study reflected the product that is sold commercially for health and fitness benefits.

Another possible factor of impact in this study could be dietary habits. We did not require subjects to keep a food log, but we set guidelines for diet within a 12-hour window of testing, including the prohibition of mouthwash, alcohol, and caffeine. Within 4 hours, no ingestion of food was allowed. However, when looking specifically at EPOC, a minimal change in the amount of ingested nitrates could show a different effect.

Additionally, a few factors regarding the exercise tests could have had an impact on results. First, the subject population recruited in this study was very ethnically diverse. The mask used for instrumentation to measure indirect calorimetry fit each individual differently and did not create an effective seal with some of the subjects, whose data were omitted from analysis limiting the number of subjects included in the analysis. Further testing of a more homogenous set of subjects may yield more accurate results. Further, an electronically-braked ergometer was not used for testing. Although subjects were verbally encouraged to maintain a specific cadence during testing and a metronome was set at the cadence with which they had to match, some variation in revolutions per minute (rpm) occurred throughout.

Lastly, this study was of a small scale and pilot in nature. Some of our statistical analyses were underpowered (0.2-0.3) and thus we cannot completely ensure that we did not make a type II error in our analyses. However, similar studies have previously utilized small number of subjects and observed significant differences. Thus, we do not believe that our small subject number fully explains our lack of an observed effect of nitrate supplementation.

In conclusion, this pilot study indicates the use of beetroot juice as an ergogenic aid in individuals who are young, healthy, and sedentary-to-moderately active yields no change in VO_2max nor energy expenditure during bouts of maximal-effort exercise. Additionally, the supplement has no effect in submaximal exercise. Supplementing with beetroot juice may provide benefit for those who are elite and highly trained to increase exercise efficiency, but not for individuals who are only moderately active or seeking weight loss.

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