Acute Dietary Nitrate Supplementation Decreases Systolic Blood Pressure and Increases Dry Static Apnea Performance in Females

Brandon Collofello, Brian Moskalik, Eric Essick

Department of Natural Sciences, University of St. Francis, Joliet, IL, USA, 60435

ABSTRACT

Collofello BS, Moskalik B, Essick EE. Acute Dietary Nitrate Supplementation Decreases Systolic Blood Pressure and Increases Dry Static Apnea Performance in Females. JEPonline 2014;17(4):15-26. Recently, dietary inorganic nitrate has been explored as a sports nutrition supplement, and has been shown to enhance exercise performance by reducing metabolic oxygen cost and lowering blood pressure. The aim of this research was to study the effects of nitrate supplementation on cardiovascular function and duration of dry static apnea in 12 healthy females. The subjects were assigned to receive 70 ml of organic beetroot juice (BR; containing ~5.0 mmol of nitrate) and a nitrate-depleted beetroot juice placebo (PLC; ~0.003 mmol of nitrate) in a randomized, double blind, crossover design. After 2.5 hrs post-ingestion of the supplement, the subjects performed three 20-sec submaximal static apneas separated by 90 sec of rest, followed by a maximal effort apnea. The duration of maximum dry static apnea, resting systolic pressure (SP), resting heart rate (HR) were statistically analyzed. The BR supplementation significantly decreased resting SP compared to the nitrate PLC conditions (x̄ = -4.82%, SD ± 4.06, Binomial test<sub>0.50</sub> P=0.039). Although a trend was noted for an increase in the BR maximal apnea duration (52.6 sec) versus the PLC (48.7 sec), the difference was not statistically significant (Binomial test<sub>0.50</sub> P=0.098). These results indicate that acute dietary nitrate decreases resting SP, but does not increase maximum static apnea in young healthy females. These findings may be advantageous for hypoxia associated athletic activities such as swimming and exercising at high altitude.

Key Words: Apnea, Blood Pressure, Nitrate, Exercise Performance
INTRODUCTION

Ingested inorganic nitrate (NO$_3^-$) is thought to be metabolized to bioactive nitrite and eventually into nitric oxide (NO), contributing to vasodilation and enhanced blood flow and oxygen delivery to metabolically active tissues (3,15,33). Furthermore, dietary nitrate is thought to increase nitric oxide bioactivity/bioavailability by increasing endogenous vascular nitric oxide generation and decreasing reactive oxygen species and inflammation, both of which appear to have cardiovascular preserving effects (33). Nitric oxide has also been reported to promote smooth muscle cell production and growth, stimulate leukocyte migration, platelet motion and accumulation, and increase expression of particular adhesion molecules (33). Furthermore, nitrate supplementation may also help improve symptoms against peripheral arterial disease (PAD) and other cardiovascular diseases (5,17,24,25,34,38).

Dietary inorganic nitrate sources commonly come from plant foods, particularly vegetables such as beets, spinach, rucola, lettuce, green beans, and a few fruits. It has been well documented that nitrate rich beet root juice (BR) is a safe and significant source of nitrate that increases plasma nitrite (NO$_2^-$) levels (1,6,11,13,25,27,33,47,48). The acceptable daily intake (ADI) for nitrate is 3.7 mg·kg$^{-1}$ of body weight per day, approximately 222 mg·day$^{-1}$ of nitrate for a 60 kg adult (10). On average an individual eating 400 g of mixed vegetables would receive 157 mg·day$^{-1}$ of dietary nitrate, an amount that is notably lower than the suggest ADI (10). In the past few years, numerous studies have examined the cardiovascular and pulmonary effects of nitrate supplementation through nitrate-rich BR (1,2,6,11,25,27,47,48). This has been shown to reduce systolic blood pressure (SP) and to reduce maximal oxygen consumption (VO$_2$ max) while increasing exercise tolerance as compared to the nitrate-depleted beet root juice control (PLC) (4,6,8,27,31,36,45,48,49). Past studies have indicated that nitric oxide donors can improve aerobic and anaerobic tolerance during exercise in untrained or moderately trained healthy subjects but not in highly trained subjects (3,7,37). This appears to indicate that non-elite healthy subjects may benefit the most from nitric oxide donors. Furthermore, research has shown that the beneficial ergonomic effects dietary nitrate donors may be limited to submaximal aerobic activities (35).

Dry static apnea is the act of holding one’s breath in a waterless environment while remaining stationary. Because static apnea reduces oxygen availability to muscles and other tissues, it is often used as an important measure of athletic performance, particularly for open water free dive and other athletic activities where oxygen availability is limited (40,42). Because nitrate supplementation reduces oxygen requirements (i.e., consumption), the general thought may be that the longer one is able to hold his or her breath, the better that individual may perform when oxygen demand begins to exceed oxygen supply or availability (hypoxia). Interestingly, recent studies have shown that acute ingestion of dietary nitrate supplements increases dry static apnea performance in highly trained competitive breath holders (10,43). However, the exact mechanism that allows for dietary nitrate to have these effects remains unknown, but may involve a decrease in metabolic cost and oxygen consumption (20). Additionally, most studies have focused on male subjects (33). Therefore, the purpose of the present study was to examine the effects of BR on dry static apnea and blood pressure in healthy young female subjects. This is the first of its kind to specifically examine the effects of nitrate supplementation on young female subjects. It is hypothesized that the nitrate rich BR supplement will increase the duration of maximum dry static apnea and reduce the SP in the young female test subjects, similar to the findings observed in male dominated studies (10,27,48,49).
METHODS

Subjects and Group Assignments
Twelve healthy women (BMI range: 18.5 to 24.9; \( \bar{x} = 21.9 \), SD ±1.9; age 18 to 30) participated in this study. The subjects were asked to: (a) maintain their normal lifestyle throughout the duration of the study; and (b) undergo a familiarization trial to reduce test anxiety prior to data collection. The familiarization protocol was performed in the same environment using the same apnea testing as the experimental protocol except that no measurements were made and the subjects did not consume any supplement.

On the days of the experiment, the subjects were instructed to arrive in a fully hydrated and rested state at was ≥3 hours post-prandial to prevent any interference with the supplement digestion and uptake. The subjects were told to: (a) avoid strenuous exercise for the 24 hrs preceding the testing; (b) avoid any uncontrolled substances affecting experimental testing; (c) refrain from forms of caffeine and nicotine for 6 hrs and alcohol for 24 hrs before each test period as previously described (27); and (c) abstain from use of mouth wash and chewing gum 24 hrs before and throughout the experiment, given that the entero-salivary system has been found to interfere with NO metabolism (15).

Experimental protocol
Using a double-blind, randomized, crossover design, the subjects were assigned to receive a dietary supplementation with 70 ml concentrated BR (≈5.0 mmol of \( \text{NO}_3^- \)) or a nitrate-depleted beetroot juice placebo (PLC; ≈0.003 mmol of \( \text{NO}_3^- \)) on separate days. The design was randomized and coded using a computer program (Random.org) to randomly assign supplements.

The subjects consumed the assigned supplement 2.5 hrs prior to each test and at least 3 hrs post-prandial, allowing for peaked plasma \( \text{NO}_2^- \) concentrations during testing (24,33). Each subject participated in two experimental test days separated by a 2-day period where no \( \text{NO}_3^- \) supplements were ingested, allowing for any remaining \( \text{NO}_3^-/\text{NO}_2^- \) to be metabolized. The two experimental days took place at the same time of day (± 30 min). On experimental days, forced vital capacity (VC) was initially measured in the standing position using a hand-held spirometer (Microlab Spirometer, Micro Medical Ltd, Kent, UK). VC was measured three times, and the largest value was recorded. The subjects were instructed to lie down in the supine position for a 5-min rest period before completing the dry static apnea test as previously described (11). The test consisted of three 20-sec (submaximum effort) apneas. Each effort was separated by a 1.5-min rest period, followed by a 3-min rest before a final maximum effort apnea (Figure 1). All apnea testing and resting periods were performed in the supine position on a floor mattress as an additional safety precaution.

Subjects were notified at the countdown time points of 2 min, 1 min, and 10 sec prior to the max apnea bout. Without a prior hyperventilation, each subject was instructed to exhale completely, which was followed by a deep, but not maximal, inspiration to begin the apnea. Schagatay has shown this to fill 85% of vital capacity volume, which is ideal for dry apneas and reduces the chance of over filling and potential syncope due to hindered

![Figure 1. Experimental Protocol.](image)
venous return (39). During sub-maximal apneas, the subjects were given time cues and the apneas were ended by verbal command. For the maximal effort apneas, the subjects were notified when they passed 20 sec of dry apnea duration.

**Experimental Measurements**

Time of apnea duration was timed using a stopwatch. Heart rate and blood oxygen saturation (SaO₂) were recorded using LabScribe and i-Worx physiologic data acquisition systems (i-Worx Systems Inc., Dover, NH). Non-invasive resting blood pressure was measured automatically. At ~10 min before maximum apnea, an average of three readings 1 min apart was recorded using an automatic arm cuff system (Omron BP786) attached to the left arm.

**Statistical Analyses**

A binomial \( t \)-test with probability (0.50) was used to compare apnea duration and percent change of SP, DP, and MAP of the BR and PLC conditions. For all data, statistical significance was accepted at \( P \leq 0.05 \). Trends were noted at \( P < 0.10 \). The data were presented as mean ± standard deviation (SD) for \( N = 12 \) subjects. All data were tested for normality and heterogeneity of variance and normal distribution using a Shapiro-Wilk \( W \) test (\( P < 0.10 \)), which indicated a randomly sampled population.

**RESULTS**

All subjects had a calculated BMI between 18.5 and 24.9 (\( \bar{x} = 21.9 \), SD ± 1.9). None of the subjects experienced an increase in SP with BR as compared to the PLC (Table 1). There was a significant percent decrease in SP for individuals given BR as compared to subjects given PLC (\( \bar{x} = -4.82\% \), SD ± 4.06, Binomial test, \( P = 0.039 \); Figure 2). In addition, the mean SP of the subjects during BR condition (\( \bar{x} = 101.25 \) mmHg) was less than the mean SP during PLC conditions (\( \bar{x} = 106.58 \) mmHg). Relative to the PLC, BR reduced SP by 5% with a -5.3 mmHg average change in SP from PLC to BR supplement. Although not significant, mean DP and mean MAP decreased by 3.18 percent (± 7.05%) and 3.94 (± 5.04%), respectively.

**Table 1. Systolic Blood Pressures (mmHg) for BR and PLC Supplement Conditions for All Subjects with Averages and Difference between Conditions.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>SP (mmHg)</th>
<th>SP (mmHg)</th>
<th>Change (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BR Conditions</td>
<td>PLC</td>
<td>PLC-BR</td>
</tr>
<tr>
<td>1</td>
<td>94</td>
<td>96</td>
<td>-2</td>
</tr>
<tr>
<td>2</td>
<td>94</td>
<td>95</td>
<td>-1</td>
</tr>
<tr>
<td>3</td>
<td>104</td>
<td>120</td>
<td>-16</td>
</tr>
<tr>
<td>4</td>
<td>84</td>
<td>94</td>
<td>-10</td>
</tr>
<tr>
<td>5</td>
<td>101</td>
<td>101</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>111</td>
<td>116</td>
<td>-5</td>
</tr>
<tr>
<td>7</td>
<td>107</td>
<td>112</td>
<td>-5</td>
</tr>
<tr>
<td>8</td>
<td>109</td>
<td>114</td>
<td>-5</td>
</tr>
<tr>
<td>9</td>
<td>94</td>
<td>94</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>96</td>
<td>101</td>
<td>-5</td>
</tr>
<tr>
<td>11</td>
<td>109</td>
<td>115</td>
<td>-6</td>
</tr>
<tr>
<td>12</td>
<td>112</td>
<td>121</td>
<td>-9</td>
</tr>
<tr>
<td>AVG</td>
<td><strong>101.25</strong></td>
<td><strong>106.60</strong></td>
<td><strong>-5.3</strong></td>
</tr>
</tbody>
</table>
Relative to PLC, BR showed a trend to increase maximal apnea duration by 7.4% (PLC: 48.7 sec vs. BR: 52.6 sec; Binomial test $P=0.098$; Figures 3 and 4). The mean maximal apnea duration after BR supplementation ($\bar{x} = 52.58$ sec) was not significantly greater under PLC conditions ($\bar{x} = 48.75$ sec; $P \geq 0.10$). In addition, the BR supplementation showed an average increase in apnea time of 10.29% ± 15.19% compared to PLC that was not significant, $P \geq 0.10$). Finally, there was no significant difference between the average percentage change in resting HR between individuals after the BR supplementation compared to the PLC conditions.

Using repeated measures AVOVA, we showed that there were intrinsic differences between individuals (Between Subjects Effects ANOVA $F_{1,12}=123.69$, $P<0.0001$), but there was no significant change within individuals by treatment (within subjects ANOVA $F_{1,12}=3.28$, $P=0.098$). There was no significant impact on effect of order (i.e., when the subject took the supplement) on the data (Table 2; two tailed $t$-test).
Table 2. t-Ratio, DF, and P Values from Two-Tailed t-Test Evaluating Effect of Order on Maximum Apnea Duration and Festing SP, DP, HR, and MAP.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>t Ratio</th>
<th>Degrees of Freedom</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max Apnea Duration</td>
<td>0.28</td>
<td>22</td>
<td>0.78</td>
</tr>
<tr>
<td>Systolic Pressure (SP)</td>
<td>-0.12</td>
<td>21</td>
<td>0.91</td>
</tr>
<tr>
<td>Diastolic Pressure (DP)</td>
<td>-0.03</td>
<td>21</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart Rate (HR)</td>
<td>0.87</td>
<td>22</td>
<td>0.39</td>
</tr>
<tr>
<td>Mean Arterial Pressure (MAP)</td>
<td>-0.07</td>
<td>20</td>
<td>0.95</td>
</tr>
</tbody>
</table>

DISCUSSION

Our results show that beetroot juice supplementation increases maximum apnea and decreases SP compared to a placebo, thus supporting the stated hypothesis. Although the exact mechanism remains unclear, it is likely that it involves improving the efficiency of nutrient and oxygen delivery to vital organs and reducing metabolic cost. Exogenous nitrate sources are metabolized into bioactive nitric oxide, which targets vascular smooth muscle causing vasodilation that contributes to a reduction in SP (33). This has previously been demonstrated in males, where nitrate reduces SP after ingestion compared to the control (8,27,48,49). Additionally, nitric oxide may enhance nutrient and oxygen transfer to active muscle allowing for prolonged apnea and exercise. This may explain the trend for increased maximum apnea duration observed after BR supplementation (3,11,33). It is important to note that forced vital capacity was not significantly different between trials in the present study. This finding suggests there is likely a physiological mechanism behind the change in apnea, and that it is not due to a change in pre-apnea gas storage capacity.

Both animal and human studies have concluded that dietary nitrate supplementation can reduce blood pressure in both normotensive as well as hypertensive test subjects (9,46). In a study that examined the effects of nitrate supplementation in exercising rats, Ferguson and colleagues (12) showed that MAP was significantly reduced in fast twitch type II muscles compared to the control. We noted a significant reduction in SP, and a non-significant trend was noted for a decrease in MAP and DP after BR supplementation. It could be that the nitrate dose in our supplement was not high enough to cause a significant difference. In the present study, nitrate may not affect DP since the vasodilation effects of bioactive nitric oxide may only be pronounced during systole. Similar to our results, Bond Jr. and colleagues (4) showed that beetroot juice treatment decreased SP but not DP in young adult healthy females. According to a systematic review and meta-analysis of randomized clinical trials (44) over a 6 yr period, inorganic nitrate and beetroot juice consumption were associated with greater changes in SP than in DP. Gilchrist et al. (14) showed that dietary nitrate supplementation did not lower MAP or improve endothelial function in their subjects. However, the lack of response may have been due to the fact that they studied type 2 diabetic subjects.
Conversely, some studies (16,28) have found that nitrate supplements significantly lowered both DP and MAP. For instance, Hobbs et al. (16) reported that acute ingestion of bread containing beetroot lowered DP, but not SP in healthy male subjects. Their findings also showed an increase in vasodilation that was independent of the endothelium, thus suggesting an alternative mechanism. It is known that during conditions of arterial hypoxia (low oxygen), nitrites can be converted into nitric oxide, which is known to have several beneficial effects on the vascular system that includes vasodilation and angiogenesis of which can reduce MAP (20,21). There continues to be a growing amount of data that demonstrates nitrate supplementation leads to vascular smooth muscle relaxation via reduction to nitric oxide and activation of the classical NO→sGC→cGMP pathway (19,32). Therefore it can be deduced that vasodilation is playing a mechanistic role in the lowering of blood pressure after nitrate supplementation. However, further investigation is needed to investigate the specific contribution of nitrate-induced angiogenesis in blood pressure reduction.

Dietary nitrate supplementation is associated with a reduction in oxygen consumption (VO₂), which can lead to an improvement in exercise efficiency (20,30). Larsen and colleagues (30) showed that nitrate supplementation lowered VO₂ by 5% compared to control in healthy trained subjects. Furthermore, both animal and human studies (22,26) have concluded that nitric oxide is involved in regulation of VO₂, as inhibition of nitric oxide synthase (NOS) reduces nitric oxide and, subsequently, results in an increase in VO₂. This indicates that reduction of nitrates and nitrites to nitric oxide is necessary for these effects to occur. A recent study (11) suggested that the physiological cost of maintaining metabolic rate is a key limiting factor for static apnea performance, and that acute dietary nitrate supplementation may increase the duration of apnea by reducing metabolic cost and VO₂. Although the exact mechanism is unknown, the nitrate/nitrite/nitric oxide reduction pathway appears to be enhanced during conditions of hypoxia, thus leading to a reduced ATP cost during muscular contraction, improved mitochondrial efficiency, and enhanced mitochondrial synthesis (18,20,27,29).

There has been some controversy on whether the ergonomic benefits of nitrate supplementation are observed more in lower intensity exercise compared to high intensity exercise. For example, Martin and colleagues (35) showed that dietary nitrate had no beneficial effects on performance in a repeated sprint test in team-sport athletes. Because VO₂ is near a maximal rate during high intensity exercise, it might be inferred that nitrate’s ergonomic benefits are only effective when oxygen availability is low. This is supported by the fact that nitrate and nitrite reduction to nitric oxide is enhanced during hypoxia (20).

Despite the significant research on nitrate supplementation, female and elderly populations have continually been underrepresented or absent in the majority of the studies. With most of the experimental designs focusing on young, athletic, male populations, or involving only a few female subjects, often there is no distinction between sexes provided in the results or explanation in the discussion. As a result, numerous conclusions have been stated based on averaging the results over subject groups, leading one to make potentially biased and faulty generalizations about parallel effects of nitrate on females despite data from male dominated experiment designs.

Faulty generalizations, in the sense that if the effects between sex groups are not identically correlated, BR effects may not have the same beneficial effects, or have an attenuated effect, on women as compared to men. Coles and Clifton (8) showed that females given a dietary nitrate supplement had an average 2.5 mmHg decrease in their SP compared to an average of 4.7 mmHg in male subjects (P<0.05) at 6 hrs post-supplement ingestion. The authors stated the differences are unclear, but maybe due to older ages in the female group, medication interference, and/or differences between sexes. Nonetheless, the attenuated decrease in SP observed in females provides
preliminary evidence for a difference in nitrate supplementation effects on blood pressure between sexes.

CONCLUSIONS

Based on this present study, nitrate supplementation might offer a way to improve exercise performance by decreasing SP and increasing apnea duration in untrained healthy and young females. Although additional research is necessary, nitrate supplementation may enhance performance ability for females in sports where VO$_2$ may be restricted, such as during swimming and anaerobic exercises. It should also be noted that the experiments by Engan et al. (11) were done on subjects who were trained for breath-hold diving, and the results showed a significant increase in their maximal apnea durations during BR compared to PLC. Future studies should consider doing a comparative study with an increased the sample size and an equal number of males and females. Additionally, research that examines the effects of nitrate supplements is of considerable interest since currently little research has been done in that area (33). Lastly, finding an optimal safe and efficacious dose may provide more significant data that may identify effects of nitrate supplementation on apnea performance and blood pressure in females.

Address for correspondence: Eric Essick, PhD, Department of Natural Sciences, University of St. Francis, Joliet, IL, USA, 60435, Email: eessick@stfrancis.edu

REFERENCES


**Disclaimer**
The opinions expressed in *JEPonline* are those of the authors and are not attributable to *JEPonline*, the editorial staff or the ASEP organization.