Dietary beetroot juice – effects on physical performance in COPD patients: a randomized controlled crossover trial

Anne Louise Friis1,*, Carina Bjornskov Steenholt1,*, Anders Løkke2, Mette Hansen1

1Section for Sport Science, Department of Public Health, Aarhus University, Denmark; 2Department of Clinical Medicine, Aarhus University, Denmark

*These authors contributed equally to this work

**Background and objective:** Dietary beetroot juice (BR) supplementation has been shown to reduce the oxygen (O2) consumption of standardized exercise and reduce resting blood pressure (BP) in healthy individuals. However, the physiological response of BR in chronic obstructive pulmonary disease (COPD) remains controversial. The objective was to test exercise performance in COPD, supplementing with higher doses of BR for a longer duration compared to previous trials in this patient group.

**Methods:** Fifteen COPD patients consumed concentrated BR (2×70 mL twice daily, each containing 300 mg nitrate) or placebo (PL) (2×70 mL twice daily, nitrate-negligible) in a randomized order for 6 consecutive days. On day 7, participants consumed either BR or PL 150 min before testing. BP was measured before completing 6-minute walk test (6MWT) and two trials of submaximal cycling. The protocol was repeated after a minimum washout of 7 days.

**Results:** Plasma nitrite concentration was higher in the BR condition compared to PL (P<0.01). There was no difference between the BR and PL conditions regarding the covered distance during the 6MWT (mean ± standard error of the mean: 515±35 m (BR) vs 520±38 m (PL), P=0.46), O2 consumption of submaximal exercise (trial 1 P=0.31 vs trial 2 P=0.20), physical activity level (P>0.05), or systolic BP (P=0.80). However, diastolic BP (DBP) was reduced after BR ingestion compared to baseline (mean difference: 4.6, 95% CI: 0.1–9.1, P<0.05).

**Conclusion:** Seven days of BR ingestion increased plasma nitrite concentrations and lowered DBP in COPD patients. However, BR did not increase functional walking capacity, O2 consumption during submaximal cycling, or physical activity level during the intervention period.

**Keywords:** nitric oxide, chronic obstructive pulmonary disease, exercise, nitrate, beetroot juice, blood pressure, nitrite

**Introduction**

Chronic obstructive pulmonary disease (COPD) patients often experience shortness of breath, which might limit their daily physical activity level (PAL) compared with healthy age-matched controls.1-3 These symptoms, along with mitochondrial dysfunction and reduced lung diffusion capacity, might contribute to a sedentary life. PA reductions may contribute to exhaustion, and in avoidance, PAL may further decrease.2,4-6

Beetroot contains high quantities of nitrate (NO3−).7 Dietary NO3− supplementation has shown to improve efficiency of energy production per unit oxygen (O2), by limiting proton leakage in the respiratory chain, thus improving the mitochondrial respiration.8-10 Hypothetically, this will decrease exercise O2 consumption in COPD patients and improve PAL.

Nitric oxide (NO) mainly facilitates the effects on the mitochondrial respiration11 and is important for smooth muscle cell relaxation in the arterial walls,12 blood
pressure (BP), and skeletal muscle contraction. NO is mainly synthesized endogenously through an ongoing process, which is dependent on nitric oxide synthase (NOS) and O₂. However, NO can additionally be synthesized from an O₂-and NOS-independent pathway. 

Exogenous dietary NO₃⁻ from beetroot juice (BR) is reduced to nitrite (NO₂⁻) by facultative bacteria in the oral cavity, followed by NO formation by the acidic stomach environment. Hypoxemia and acidosis facilitate the NO₃⁻→NO₂⁻→NO pathway, thereby complementing the classical pathway, during exercise. Ingestion of NO₃⁻-rich vegetables will, therefore, contribute to an increased NO₃⁻ and NO₂⁻ concentration in the bloodstream. 

BR supplementation has shown to reduce O₂ consumption during standardized exercise in healthy individuals, and increase exercise performance in COPD patients and peripheral arterial disease (PAD). Therefore, BR supplementation might improve exercise capacity in individuals with reduced O₂ consumption.

Studies demonstrate an improved exercise performance following BR supplementation in COPD patients, although lacking an optimal placebo (PL). Later, a study using ideal PL but suboptimal dose, was not able to observe improvements in O₂ consumption during exercise, walking performance, or BP.

The purpose of this study is to evaluate exercise performance using the 6-minute walk test (6MWT), supplementing with higher doses of dietary NO₃⁻ as BR for 7 days; secondarily, to evaluate the O₂ consumption of submaximal cycling, amendments in BP, and PAL.

**Methods**

**Participants**

Fifteen COPD patients completed the trial. Inclusion criterion was moderate-severe COPD (forced expiratory volume in 1 s [FEV₁] <80% of predicted), in accordance with criteria of the American Thoracic Society. Exclusion criteria included smoking, failure to complete physical testing, ongoing participation in rehabilitation programs, pacemaker, or use of nicotine products, oxygen mask, beta blockers, antibacterial mouthwash, chewing gum, or stomach-neutralizing medicine during interventions. Participants were recruited from the local hospital, by advertising (http://sundhed.dk), and through telephone calls. All data were collected at the Section of Sport Science, Department of Public Health, Aarhus University. The study was approved by The Central Denmark Region Committees on Health Research Ethics (1–10-72-13-13) and complied with the Declaration of Helsinki. All participants received oral and written information about the study and gave written informed consent prior to inclusion. The study was registered at ClinicalTrials.gov on January 13th, 2017, with the identification number NCT03020862.

Previous trials in healthy individuals have observed significant effects on exercise capacity and physical responses during exercise in randomized controlled crossover trials including only nine individuals or eight individuals and using a dose three times lower per day than in the present trial. Nevertheless, we were aiming to achieve 15 completers because we expected that the variation in response might be greater in the COPD patients.

**Design**

This randomized, double-blinded, PL-controlled, crossover study investigated the acute and accumulated effect of BR supplementation in COPD patients. The participants completed four visits: screening, habituation, and experimental days at the end of each intervention. They consumed 2×70 mL concentrated BR (Beet-it; James White Drinks, Ipswich, UK, 300 mg NO₃⁻) or PL (Beet-it; James White Drinks; NO₃⁻-negligible) each morning and night for six days. During this period, PAL was estimated by accelerometry. The experimental day, outlined in the following sections, was carried out on day 7 (Figure 1).

**Study procedure**

Participants were randomly allocated to BR or PL using an unrestricted computer-generated sequence, only available to laboratory technicians not involved in the project, except for packaging of the products. PL was an organic NO₃⁻-depleted BR, similar in taste and appearance.

To standardize the NO₃⁻ intake, participants were instructed to avoid variation in habitual diet, limit intake of NO₃⁻-rich vegetables, and avoid alcohol and strenuous activities for 24 hours and caffeine for 12 hours before testing. Participants arrived in the morning after overnight fasting and consumed a standardized meal, according to 20% of their individual estimated daily energy requirement.

At screening, height, weight, and BP were measured, and the participants completed a 6MWT and Aastrand–Rhyming submaximal cycle test (Figure 1). Participants were familiarized with the equipment at the habituation visit. Upon arrival, BP was measured after 10 min supine rest (Monitor: BP A100 Plus; Microlife, Taipei, Taiwan) and a blood sample was collected. Afterwards, 6MWT was performed followed by submaximal cycling. Immediately following the physical tests blood lactate was analyzed (1500 SPORT Lactate Analyzer; YSI Life Sciences, Yellow Springs, OH, USA).
At day 7 of the interventions, the participants arrived in the morning after overnight fasting. Details regarding specific tests and analysis are outlined below, but the experimental day consisted of BP measurements (baseline values were measured on the morning of the PL period), blood sampling, and baseline blood lactate measurements (fingerprick test). The participants consumed a standardized meal and 2×70 mL of BR/PL. After 2½ hours, another blood sample was collected and BP was measured. Participants performed a 6MWT followed by blood lactate collections and reported a value on the Borg scale for ratings of perceived exertion (Borg-RPE). After rest (30 min), another blood sample was collected following completion of submaximal cycling, blood lactate, and Borg-RPE collection (Figure 1).

**Measurements**

The 6MWT was conducted in accordance with established guidelines. Considering a possible learning effect, the test was performed at screening and habituation visit. The participants consumed a standardized meal and 2×70 mL of BR/PL. After 2½ hours, another blood sample was collected and BP was measured. Participants performed a 6MWT followed by blood lactate collections and reported a value on the Borg scale for ratings of perceived exertion (Borg-RPE). After rest (30 min), another blood sample was collected following completion of submaximal cycling, blood lactate, and Borg-RPE collection (Figure 1).

Figure 1 Schematic overview of the study protocol (top) and the experimental protocol (bottom) completed during the habituation visit and at day 7 of each intervention period (indicated by dashed arrows).

**Note:** *indicates the time when the measurements were performed.

**Abbreviations:** BP, Blood Pressure; 6MWT, 6-minute walk test; BR, Beetroot juice; PL, Placebo; Borg-RPE, Borg scale for ratings of perceived exertion; NO₂⁻, Nitrite.
Software, Pensacola, FL, USA) to store data at 80 Hz with 10 s epoch. Before analysis, data between retiring to bed and wakeup time were removed, as this was expected to be non-nawake time. When participants went to bed past midnight, the activity was included in the calculations for the previous day. To estimate intensity and duration of PA, established cutoffs for accelerometers were used. Data were considered valid when wear time was >10 h/day for ≥4 days for each intervention.

To determine plasma NO$_2^-$ concentration, ~4 mL venous blood was collected from the bilateral antecubital vein in 8 mL lithium-heparin vacutainers (ref 367378; Becton, Dickinson and Co, Franklin Lakes, NJ, USA). Samples were centrifuged at 4,000 rpm at 4°C for 10 min and plasma was frozen at −80°C until further analysis.

NO$_2^-$ concentrations were determined using chemiluminescence. Blood plasma was injected into the reaction vessel and NO$_2^-$ was reduced by $I^-_3$-reagent to gaseous NO and detected by Sievers NO analyzer (NOA, 280i; GE Analytical Instruments, Boulder, CO, USA) using helium as a carrier gas. Calibration was performed using known NO$_2^-$ amounts according to the manufacturer’s manual.

**Statistical analyses**

Statistical analyses were performed in STATA 12.1. Data for 6MWT (meters, lactate, and HR), PAL in each activity zone (% of total time), and COPD Assessment Test, and data from cycling trials (VO$_2$ steady state, lactate, HR, and Borg-RPE) were analyzed using two-tailed paired t-tests. Data for NO$_2^-$ and VO$_2$ of the submaximal cycling trials (all data points) and BP were analyzed using a two-way repeated-measurements analysis of variance. GraphPad Prism version 6.02 was used for figures. Statistical significance was accepted at $P<0.05$. Results are presented as mean ± standard error of the mean unless stated otherwise.

**Results**

Nineteen patients were included and randomized. Of these, 15 COPD patients completed the interventions between December 2013 and April 2014. The four patients who did not complete dropped out for reasons not related to the intervention beverages. The flow of participants is illustrated in Figure 2. Participants reported to comply with supplementation, although two reported nausea after ingestion of both PL (n=2) and BR (n=2), but still completed the intervention and tests. Baseline characteristics of the participants are presented in Table 1.

**Plasma nitrite concentration**

NO$_2^-$ was significantly higher in the BR condition compared to PL (BR: 538.5±186.6 nM; PL: 140.0±68.9 nM, $P<0.01$) at the end of the intervention. After PL ingestion, no change in NO$_2^-$ concentration was detected (150 min: 193.4±95.4 nM; 210 min: 192.0±124.6 nM, time and interaction $P>0.05$). However, NO$_2^-$ concentration increased after BR ingestion during the experimental day, and was significantly higher, 150 min (1,118.4±317.0 nM, P<0.01) and 210 min (1,099.2±318.3 nM, P<0.01), compared to before BR ingestion at day 7 (Figure 3).

**6MWT**

All participants (n=15) completed the 6MWT during both interventions. Three participants expressed discomfort during the test. There was no difference between conditions in the covered distance (BR: 515±35 m vs PL: 520±38 m, P=0.46; 15).

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**Table 1 Baseline characteristics of participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
<th>Range</th>
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<tr>
<td>Male gender, n (m/f)</td>
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<td>Age (years)</td>
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<td>(163–182.5)</td>
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<td>Height (cm)</td>
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<td>Weight (kg)</td>
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<td>BMI (kg/m$^2$)</td>
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<td>VO$_2$ (liters)</td>
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<td>(0.44–2.81)</td>
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<td>FEV$_1$ (% predicted)</td>
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<td>(22–76)</td>
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<tr>
<td>FVC (liters)</td>
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<td>FVC (% predicted)</td>
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<td>FEV$_1$/FVC (%)</td>
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<td>(0.31–1.05)</td>
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<tr>
<td>CAT score (n=14)</td>
<td>16.4±5.11</td>
<td>(7–22)</td>
</tr>
</tbody>
</table>

**Note:** Data are presented as mean ± SD and with data range (n=15).

**Abbreviations:** BMI, body mass index; CAT, COPD Assessment Test; FEV$_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; MRC, Medical Research Council Scale; m/f, male/female.
There was no difference in HR (BR: 119±6 bpm vs PL: 119±5 bpm, *P=0.86*), lactate concentration (BR: 2.20±0.31 mmol L⁻¹ vs PL: 2.54±0.44 mmol L⁻¹, *P=0.16*), or Borg-RPE (BR: 14±1 score vs PL: 15±1 score, *P=2.20*) between conditions.

Oxygen consumption during submaximal cycling

O₂ consumption during submaximal cycling is illustrated in Figure 5. Two participants were unable to complete both trials because of discomfort and were excluded from further analysis, resulting in a total of 13 completing the test. O₂ consumption increased during each trial (time *P<0.001*; Figure 5), but was not influenced by treatment (interaction trial 1 *P=0.31* vs trial 2 *P=0.20*).

Mean oxygen consumption (steady-state period)

When comparing conditions for both trials, there was no difference between trials (trial 1: BR: 0.96±0.12 L/min, PL: 1.01±0.12 L/min, *P=0.23* vs trial 2: BR: 0.99±0.12 L/min, PL: 1.02±0.12, *P=0.37*). There was no difference between trials (*P=0.25*), no difference as a consequence of treatment (*P=0.27*), and no significant interaction between trial and treatment (*P=0.33*).

Secondary outcomes during submaximal cycling

Lactate concentration during cycling did not differ between conditions for trials (trial 1: BR: 2.37±0.29 mmol/L, PL: 2.07±0.31 mmol/L, *P=0.10*, *n=9* paired data vs trial 2: BR: 1.98±0.38 mmol/L, PL: 1.66±0.28 mmol/L, *P=0.15*, *n=6* paired data). HR during cycling did not differ between trials for conditions (trial 1: BR: 112±4 bpm, PL: 114±5 bpm, *P=0.54*, *n=13* paired data vs trial 2: BR: 115±5 bpm, PL: 116±4 bpm, *P=0.63*, *n=13* paired data). No difference in Borg-RPE was observed during trial 1 (BR: 14±1 vs PL: 13±1, *P=0.40*, *n=15* paired data). Trial 2 Borg-RPE was significantly higher during BR compared to PL (BR: 14±1 vs PL: 13±1, *P<0.05*, *n=13* paired data).

Physical activity level

Data from four participants were excluded because of missing data. Thus, a total of 11 participants were included in the analysis. No difference between time spent in the three PALs was observed for conditions (sedentary: BR: 85%±2% vs PL: 84%±2%, *P=0.32*, light: BR: 13%±2% vs PL: 14%±2%, *P=0.18*, moderate-high intensity: BR: 2.0%±0.0% vs PL: 2.0%±0.0%, *P=0.85*). Mean wear time did not differ between conditions (BR: 15.1±0.4 h vs PL: 14.8±0.3 h, *P=0.19*), and there was no difference between the number of days data were collected (BR: 5.6±0.2 days vs PL: 5.8±0.2 days, *P=0.51*).

Blood pressure

Measures of systolic BP (SBP) and diastolic BP (DBP) for all participants (*n=15*) are included in the BP analysis.
SBP was significantly lower 150 min after PL ingestion compared to arrival at the lab (0 min: 129±4 mmHg and 150 min: 122±4 mmHg, P<0.05). SBP was reduced 150 min after BR ingestion (0 min: 129±3 mmHg and 150 min: 122±4 mmHg, P<0.05). The reduction in SBP was not influenced by treatment (interaction P=0.80; Figure 6).

DBP was significantly lower 150 min after ingestion of beverages compared to arrival at the lab (time P=0.03). The reduction was significant after BR ingestion compared to baseline (mean difference: −4.6, 95% CI: −9.1 to −0.1, P<0.05), but not after PL ingestion (mean difference: −3.1, 95% CI: −7.6 to −1.4, P=0.24; Figure 6). No significant interaction was observed (P=0.56; Figure 6).

Discussion
BR intake (2×140 mL/day) for 7 days, and just before testing, did not increase functional capacity and PAL or reduce $O_2$ consumption of submaximal cycling. However, DBP was reduced 150 min following BR ingestion compared to baseline.

BR contains $NO_3^−$, which after conversion to $NO_2^−$ should introduce physiological beneficial effects in COPD patients. We did not directly measure $NO_3^−$ concentration in the batch, but based on prior studies, the content of 2×70 mL aliquots is ~0.6 mg (7.6–9.6 mmol). As expected, plasma $NO_3^−$ concentration significantly increased following BR administration, although with large variations between participants. Thus, the concern regarding the age-related heterogeneity of COPD patients. Our results, showing no significant effect of BR on PAL, walking performance, and $O_2$ consumption may, therefore, partly be explained by non-responders (six of 15 reached $NO_2^−$ levels >1,000 nM). Our study was not statistically powered to perform subanalyses, merely including patients having a marked increase in plasma $NO_3^−$. Hence, future investigations should try identifying specific COPD phenotypes obtaining high plasma $NO_3^−$ levels after BR ingestion and who, with higher probability, could benefit physiologically. In addition, the trial was not designed for performing stratified analyses depending on degree of

**Figure 5** Oxygen consumption during submaximal cycling (entire period).

*Notes:* Data are presented as mean ± standard error of the mean ($n=13$). (A) Trial 1 (interaction $P=0.03$; time $P<0.001$) and (B) trial 2 after 10 min break (interaction $P=0.20$; time $P<0.001$). ***indicates a significant increase in oxygen consumption during the trial ($P<0.001$).
Nevertheless, we repeated the statistical analysis for the primary outcome parameter 6MWT separately, based on data from the group of patients with an FEV\textsubscript{1} < 50% (n=5) and in the group of patients with an FEV\textsubscript{1} > 50%. The walking distance was not different between the two stratified groups of COPD patients. Furthermore, no significant effect of BR was observed in either of the groups.

Dietary NO\textsubscript{3} has shown to optimize mitochondria efficiency and blood flow in young healthy participants.\textsuperscript{9,32} BR reduced O\textsubscript{2} consumption in healthy participants by 10% and 19% during submaximal exercise.\textsuperscript{16,18} To the contrary, we did not observe reduced O\textsubscript{2} consumption during submaximal exercise even though we used equal or higher doses and longer supplementation periods.\textsuperscript{16,18} The lacking physiological effects were underlined by significantly higher Borg-RPE ratings during BR supplementation. Our findings are supported in a recent study\textsuperscript{20} investigating the effect of BR in COPD, which failed to detect a significant O\textsubscript{2} reduction during submaximal cycling. Thus, we cannot exclude that age/disease-related causes might reduce the responsiveness to BR supplementation.

COPD patients are susceptible to weather factors, which are conveyed as impaired respiration, potentially impacting cycling and 6MWT considerably. Two participants failed to complete cycling because of respiration problems, during the PL condition. The susceptibilities might attenuate measurability of the effect of BR supplementation in COPD, and our study might be statistically underpowered because of greater day-to-day variation in this group compared to healthy individuals.

Distance covered in the 6MWT and the secondary outcomes were not changed by BR ingestion, although an improved maximal walking performance in PAD patients following acute NO\textsubscript{3} supplementation has been observed.\textsuperscript{19} Nevertheless, similar to our observation, no change in the 6MWT compared to PL has been reported in COPD\textsuperscript{20} and in older healthy adults;\textsuperscript{33} which was attributed to a compromised ability of older individuals to perform maximally.\textsuperscript{33} Although lactate and HR following 6MWT were enhanced compared to rest in the present study, the increases were minor and not different than after cycling. These observations...
Submaximal cycling was an uncommon activity for the majority of the COPD patients. Although the habituation was completed to eliminate a learning effect, the participants did not become familiar with cycling, thus potentially affecting respiration during testing. Taking the learning effect into consideration, performing two 6MWT, and selecting the best distance, thus omitting submaximal cycling, might be a more suitable approach in future research on physical performance in this patient group.

**Conclusion**

BR supplementation increased plasma NO$_2^-$ concentration and lowered DBP in moderate-severe COPD patients. However, BR supplementation two times/day for 7 days did not increase functional walking capacity or PAL during the intervention. Submaximal cycling O$_2$ consumption was not significantly influenced by BR supplementation. Further studies are warranted to clarify the therapeutic role of BR supplementation in COPD patients.

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**Disclosure**

The authors report no conflicts of interest in this work.

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