

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

Anne Louise Friis^{1,*}
Carina Bjørnskov
Steenholt^{1,*}
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 respiration⁹ and is important for smooth muscle cell relaxation in the arterial walls,¹¹ blood

Correspondence: Mette Hansen
Section for Sport Science, Department of Public Health, Aarhus University, Dalgas Avenue 4, 8000 Aarhus, Denmark
Email mhan@ph.au.dk

pressure (BP),¹² and skeletal muscle contraction.¹³ 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.¹⁰

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.¹⁰ Hypoxemia and acidosis facilitate the NO₃⁻-NO₂⁻-NO pathway, thereby complementing the classical pathway, during exercise.¹⁰ Ingestion of NO₃⁻-rich vegetables will, therefore, contribute to an increased NO₃⁻ and NO₂⁻ concentration in the bloodstream.^{14,15}

BR supplementation has shown to reduce O₂ consumption during standardized exercise in healthy individuals,¹⁶⁻¹⁸ and increase exercise performance in COPD patients^{14,15} and peripheral arterial disease (PAD).¹⁹ 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^{14,15} 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.²⁰

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.²¹ 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 individuals¹⁷ or eight individuals¹⁶ 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.²²

At screening, height, weight, and BP were measured, and the participants completed a 6MWT and Astrand-Rhyming submaximal cycle test (Figure 1).²³

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).

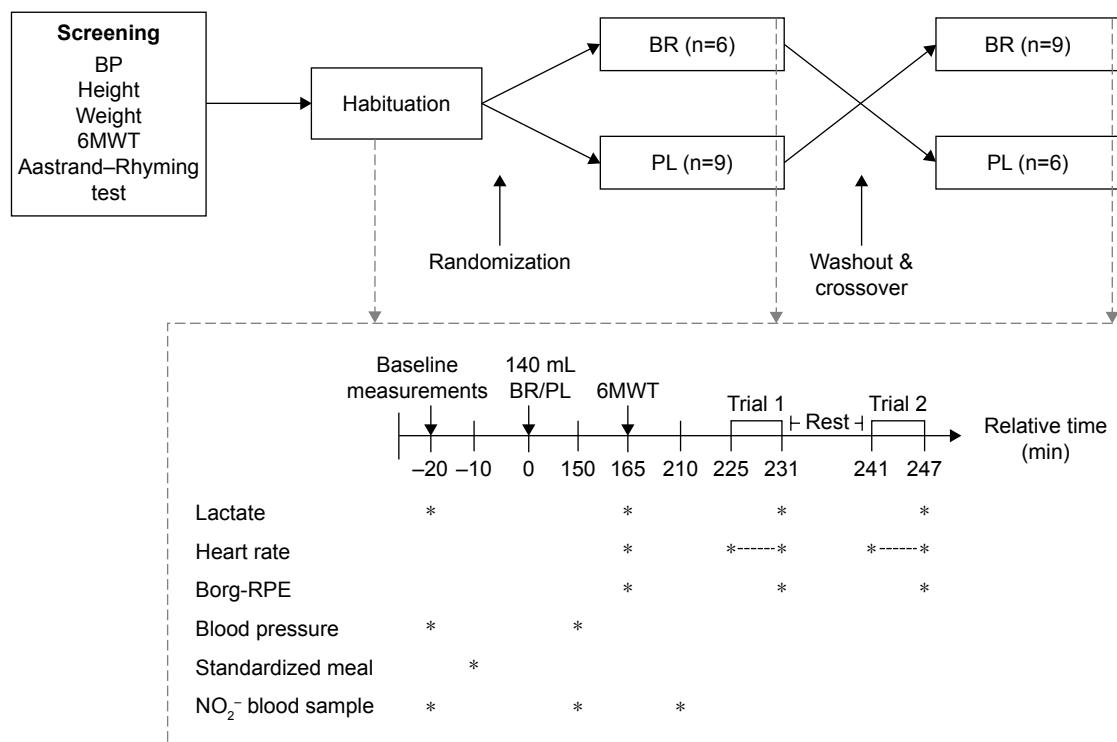


Figure 1 Schematic overview of the study protocol (top) and the experimental protocol (bottom) completed during the habituation visit and at day 7 of each intervention period (indicated by dashed arrows).

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.

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 followed by completion of submaximal cycling, blood lactate, and Borg-RPE collection (Figure 1).

Measurements

The 6MWT was conducted in accordance with established guidelines.²⁴ Considering a possible learning effect, the test was performed at screening and habituation visit.^{24,25}

The 2×6 min standardized submaximal cycling test with a constant load (60 rpm) and a 10 min break between trials was performed at each experimental day. Respiratory measurements (AMIS 2001; Innovision, Odense, Denmark) and heart rate (HR) (POLAR RS800CX) were continuously

measured. Before testing, the analyzer was calibrated using known O₂ concentrations and CO₂ concentrations. The same cycle ergometer (Monark 894E) was used for all tests and the submaximal load was equivalent to 50% of the estimated Watt_{max}. Ending each trial, HR, lactate concentration, respiratory measures, and Borg-RPE were registered. Mean VO₂ values in the last 2 min were considered to reflect steady state and were used for analysis of the effect between interventions in addition to analysis for differences over the entire cycling period of each trial.

The Aastrand–Rhyming test was conducted to ensure participants' ability to cycle continuously for 10 min and to estimate Watt_{max},²³ which was used for submaximal cycling at the intervention. The workload on the Aastrand–Rhyming test was adjusted so that the participants could cycle for 6 min at an HR between 105 and 115 bpm followed by 4 min with an HR between 125 and 140 bpm. HR and workload were registered every minute until steady-state HR (varied <3–5 bpm). Following the test, HR_{max} (220 bpm–age) and Watt_{max} were calculated.²³

Daily PAL was monitored using accelerometry (ActiGraph wGT3X-BT) during waking hours (except water activities), on the right hip all 6 intervention days, except on experimental days. The accelerometer was programmed (ActiLife Analysis

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-awake 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.²⁶ 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_3^- 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_3^- 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 \pm 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

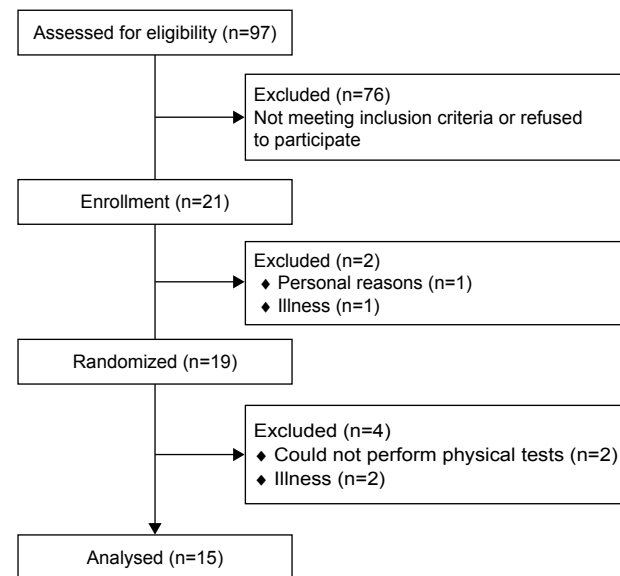


Figure 2 Flow of participants including enrollment, randomization, and completion.

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 ($1,118.4 \pm 317.0$ nM, $P < 0.01$) and 210 min ($1,099.2 \pm 318.3$ 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, $P = 0.46$;

Table 1 Baseline characteristics of participants

Characteristics	Mean \pm SD	Range
Male gender, n (m/f)	9/6	
Age (years)	63 \pm 13	(27–77)
Height (cm)	171.8 \pm 6.4	(163–182.5)
Weight (kg)	75.8 \pm 17.3	(51–112.6)
BMI (kg/m ²)	25.5 \pm 4.8	(19.2–34.8)
FEV ₁ (liters)	1.4 \pm 0.7	(0.44–2.81)
FEV ₁ (% predicted)	44.7 \pm 15.1	(22–76)
FVC (liters)	2.7 \pm 0.7	(1.37–4.25)
FVC (% predicted)	74.2 \pm 14.7	(49–97)
FEV ₁ /FVC (%)	0.61 \pm 0.19	(0.31–1.05)
MRC score	2.47 \pm 0.74	(1–4)
CAT score (n=14)	16.4 \pm 5.11	(7–22)

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

Abbreviations: BMI, body mass index; CAT, COPD Assessment Test; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; MRC, Medical Research Council Scale; m/f, male/female.

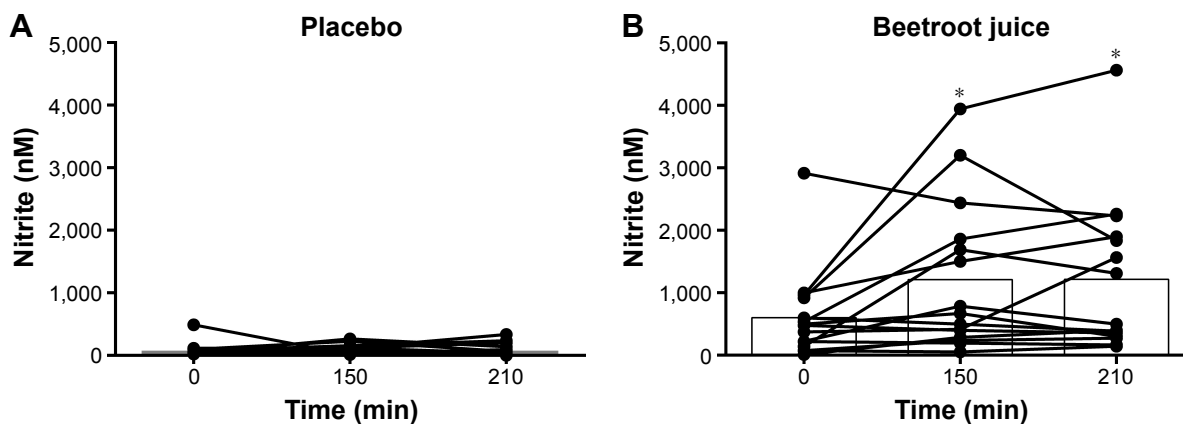


Figure 3 Individual and mean plasma nitrite (nM) at the experimental day at arrival (0 min), 150 and 210 min after ingestion of either (A) placebo (PL) or (B) beetroot juice (BR). Nitrite was significantly higher in the BR period compared to PL ($P<0.01$), and nitrite increased after ingestion of BR, but not after PL (time $P<0.01$, interaction $P<0.05$). **Note:** * $P<0.01$, nitrite higher at 150 and 210 min after ingestion of BR compared to arrival (0 min).

Figure 4). 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: $85\%\pm 2\%$ vs PL: $84\%\pm 2\%$, $P=0.32$, light: BR: $13\%\pm 2\%$ vs PL: $14\%\pm 2\%$, $P=0.18$, moderate-high intensity: BR: $2.0\%\pm 0.0\%$ vs PL: $2.0\%\pm 0.0\%$, $P=0.85$). 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.

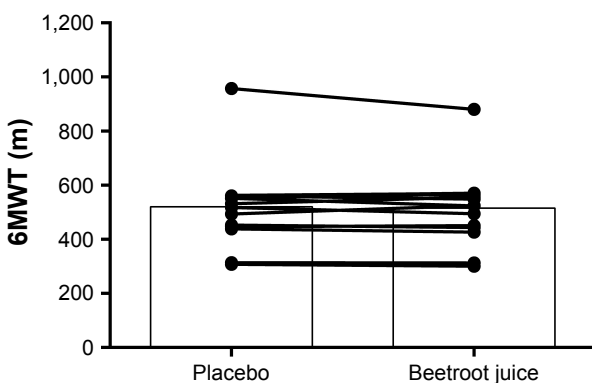


Figure 4 Individual and mean distance covered in meters during 6MWT. **Note:** Data are presented as mean \pm standard error of the mean ($n=15$). **Abbreviation:** 6MWT, 6-minute walk test.

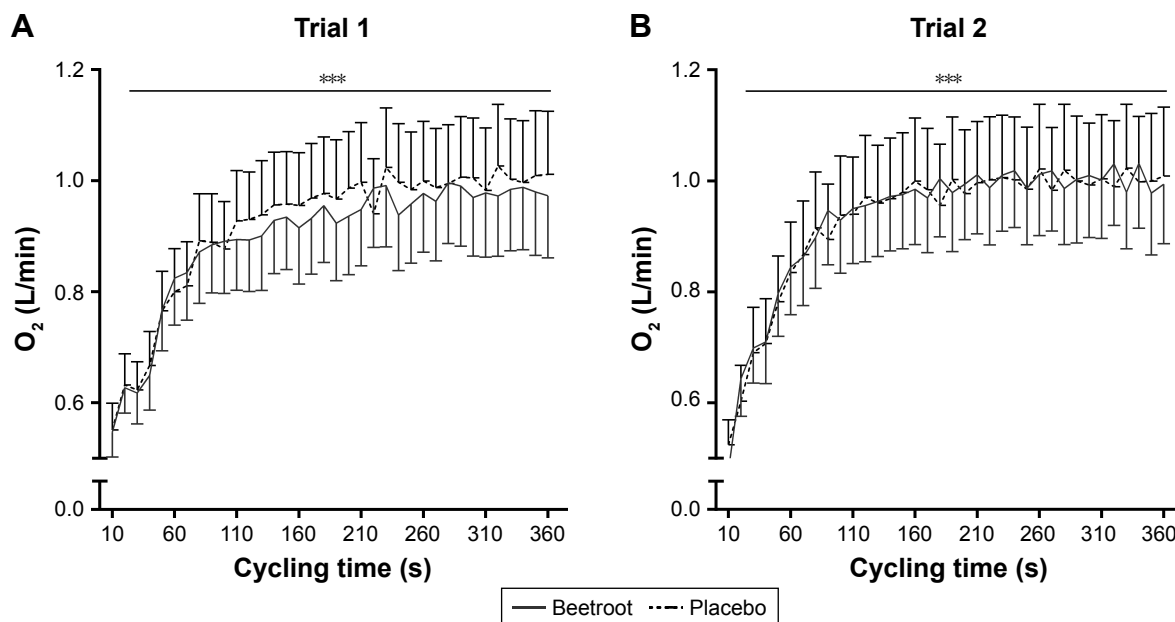


Figure 5 Oxygen consumption during submaximal cycling (entire period).

Notes: Data are presented as mean \pm standard error of the mean ($n=13$). **(A)** Trial 1 (interaction $P=0.31$; 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 ($P<0.001$).

SBP was significantly lower 150 min after PL ingestion compared to arrival at the lab (0 min: 129 ± 4 mmHg and 150 min: 121 ± 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 – (-0.1) , $P<0.05$), but not after PL ingestion (mean difference: -3.1 , 95% CI: -7.6 – 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,^{15,27} the content of 2×70 mL aliquots is ~ 0.6 mg (7.6 – 9.6 mmol). As expected, plasma NO_2^- concentration significantly increased following BR administration, although with large variations between participants. Thus, the concern regarding the age-related

changes in gastrointestinal function and facultative bacteria utility, potentially impairing the formation of NO_2^- , remains unresolved. Plasma NO_2^- concentration following BR supplementation was similar to the findings in PAD patients¹⁹ and COPD patients^{14,15} using similar doses. Using smaller dosing regimens, the plasma NO_2^- concentration was additionally comparable to that in active young men, indicating that COPD patients might have an impaired capacity for the conversion of dietary ingested NO_3^- to NO_2^- compared to healthy individuals.^{13,28–31}

To our knowledge, participants in the present study ingested the highest dose used in studies including COPD patients. This protocol was chosen because an accumulated effect of BR in reducing the O_2 consumption of submaximal exercise of healthy individuals has been observed.^{16,18,28} Heterogeneous plasma NO_2^- responses to BR supplementation were observed in the present study, which may reflect the heterogeneous nature 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 subanalysis, merely including patients having a marked increase in plasma NO_2^- . Hence, future investigations should try identifying specific COPD phenotypes obtaining high plasma NO_2^- 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

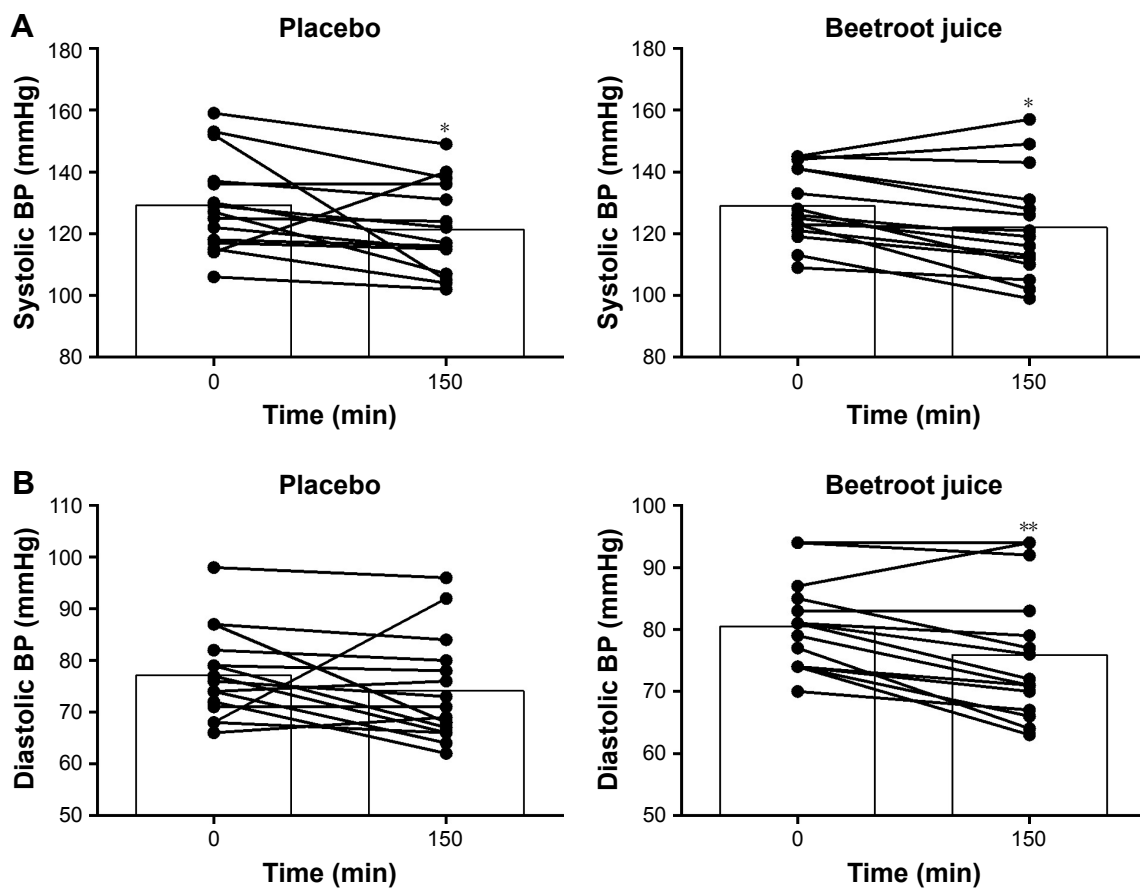


Figure 6 Individual and mean response in (A) systolic blood pressure (mmHg) and (B) diastolic blood pressure (mmHg) (n=15).

Notes: * $P < 0.01$ denotes difference between baseline and 150 min after ingestion of both placebo and BR. ** $P < 0.05$ denotes difference between baseline and 150 min after ingestion of BR.

Abbreviations: BP, blood pressure; BR, beetroot juice.

COPD. Nevertheless, we repeated the statistical analysis for the primary outcome parameter 6MWT separately, based on data from the group of patients with an $FEV_1 < 50\%$ (n=5) and in the group of patients with an $FEV_1 > 50\%$. 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_3^- has shown to optimize mitochondria efficiency and blood flow in young healthy participants.^{9,32} BR reduced O_2 consumption in healthy participants by 10% and 19% during submaximal exercise.^{16,18} To the contrary, we did not observe reduced O_2 consumption during submaximal exercise even though we used equal or higher doses and longer supplementation periods.^{16,18} The lacking physiological effects were underlined by significantly higher Borg-RPE ratings during BR supplementation. Our findings are supported in a recent study²⁰ investigating the effect of BR in COPD, which failed to detect a significant O_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_3^- supplementation has been observed.¹⁹ Nevertheless, similar to our observation, no change in the 6MWT compared to PL has been reported in COPD²⁰ and in older healthy adults;³³ which was attributed to a compromised ability of older individuals to perform maximally.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

are questionable as to whether the 6MWT reflects maximal performance. The reduced ability to perform maximally may partly be caused by other health-related challenges, as three of 15 participants reported having knee pains unrelated to COPD. In support, a negative correlation between pain severity and 6MWT distance has been reported.⁴

When testing COPD patients on a shuttle walk test (SWT) following an acute consumption of BR, an 11% increase in walking distance was observed ($P=0.005$).¹⁴ However, these results remain questionable, as a true PL was lacking and the energy content of the drinks was unequal.¹⁴ Conversely, following a 3-day BR supplementation and using a lower BR dose, Leong et al³⁴ failed to observe a significant increase in walking distance or time-to-fatigue, thereby questioning the superiority of the SWT compared to the 6MWT in this patient group.

PAL measured in the present study corresponded with the previous measurements in COPD patients.³⁵ Previous studies show that the effect of NO_3^- supplementation, if continuously supplemented, is maintained for 6–15 days in healthy individuals.^{16,18,28} Therefore, we hypothesized that COPD patients would increase PAL in the BR period, and unfortunately this was not observed. This is not surprising, as O_2 consumption during cycling was not reduced, and we cannot exclude that PAL is primarily based on scheduled habitual activity plans instead of small variations in physical capacity. The accelerometer might not be sensitive in detecting small changes in this patient/age group, partly explaining the lack of effect on PAL.

BR supplementation reduced DBP compared to baseline, which was not observed in the PL condition. This observation agrees with the observations in healthy older adults,³³ PAD patients,¹⁹ and COPD patients.^{14,15,35} In contrast to these findings,^{14,15,33} we did not observe a reduction in SBP following NO_3^- supplementation. Another study in COPD failed to show a reduction in both SBP and DBP,²⁰ which may have been due to a lower BR dose compared with the present and previous studies in COPD patients.^{14,15}

Consistent with the previous findings, we observed large variations in response to BR supplementation in COPD patients^{14,15,27} compared to healthy controls.³¹ We included a small heterogeneous COPD group, which potentially limits the statistical power for detecting small, but relevant, differences. The included group largely reflects the nature of COPD patients, adding to the external validity of the results. However, future research in this area should try to identify specific COPD phenotypes and include a larger and more homogeneous group, with respect to age, body mass index, and severity of their COPD, thus increasing the internal validity.

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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