Dietary nitrate supplementation to enhance exercise capacity in hypoxic COPD: EDEN-OX, a double-blind, placebo-controlled, randomised cross-over study

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ABSTRACT
Rationale Dietary nitrate supplementation improves skeletal muscle oxygen utilisation and vascular endothelial function. We hypothesised that these effects might be sufficient to improve exercise performance in patients with COPD and hypoxia severe enough to require supplemental oxygen.

Methods We conducted a single-centre, double-blind, placebo-controlled, cross-over study, enrolling adults with COPD who were established users of long-term oxygen therapy. Participants performed an endurance shuttle walk test, using their prescribed oxygen, 3 hours after consuming either 140 mL of nitrate-rich beetroot juice (BRJ) (12.9 mmol nitrate) or placebo (nitrate-depleted BRJ). Treatment order was allocated (1:1) by computer-generated block randomisation.

Measurements The primary outcome was endurance shuttle walk test time. The secondary outcomes included area under the curve to isotime for fingertip oxygen saturation and heart rate parameters during the test, blood pressure, and endothelial function assessed using flow-mediated dilatation. Plasma nitrate and nitrite levels as well as FE\textsubscript{O2} were also measured.

Main results 20 participants were recruited and all completed the study. Nitrate-rich BRJ supplementation prolonged exercise endurance time in all participants as compared with placebo: median (IQR) 194.6 (147.5–411.7) s vs 159.1 (121.9–298.5) s, estimated treatment effect 62 (33–106) s (p<0.0001). Supplementation also improved endothelial function: NR-BRJ group +4.1% (−1.1% to 14.8%) vs placebo BRJ group −5.0% (−10.6% to −0.6%) (p=0.0003). Conclusion Acute dietary nitrate supplementation increases exercise endurance in patients with COPD who require supplemental oxygen.

Trial registration number ISRCTN14888729.

INTRODUCTION
People with COPD may develop hypoxaemia as the condition becomes more severe, impacting on their ability to perform day-to-day activities. Mechanisms include ventilation perfusion mismatch, reduced cardiac output due to hyperinflation and pulmonary vascular limitation, as well as reduced muscle efficiency. In individuals who are sufficiently hypoxaemic, long-term oxygen therapy (LTOT) improves survival, and in many individuals ambulatory oxygen therapy (AOT) improves exercise performance.

Nitric oxide (NO) has potential as a modulator of exercise performance. A ubiquitous signalling molecule, NO is involved in a number of processes including ventilation perfusion mismatch, as well as mitochondrial and cellular respiration, glucose uptake into the skeletal muscle, skeletal muscle contraction, neurotransmission and fatigue development. NO is produced both by oxygen-dependent NO synthases catalysing its production from L-arginine and an alternative nitrate (NO\textsubscript{3}−)–nitrite (NO\textsubscript{2}−)–NO pathway. The latter can be influenced by supplementation with exogenous dietary NO\textsubscript{3}− and is enhanced in conditions of hypoxia and low pH as found in exercising skeletal muscle.

Dietary NO\textsubscript{3}− supplementation has been shown to reduce the oxygen cost of exercise in healthy individuals in normoxic conditions and in conditions of hypoxia. Recently our research group has shown that it augments improvement in exercise capacity seen in people with COPD following pulmonary rehabilitation. We have also previously shown that dietary NO\textsubscript{3}− supplementation reduces the oxygen cost of exercise during
endurance cycle ergometry in COPD. However, that study, which excluded patients who required supplemental oxygen, did not demonstrate an improvement in exercise capacity.

The aim of the present study was therefore to assess the acute effect of dietary supplementation in the form of nitrate-rich beetroot juice (NR-BRJ) on exercise performance in individuals with COPD who require supplemental oxygen on exertion, hypothesising that this would increase exercise capacity, measured as endurance shuttle walk time (ESWT), as well as improve endothelial function in people with this specific phenotype.

MATERIALS AND METHODS

Study design

The EDEN-OX (Effect of Dietary Nitrate Supplementation on Exercise Performance in Hypoxia) study was a single-centre, double-blind, placebo-controlled, randomised cross-over trial comparing the effects of dietary NO$_3^-$ supplementation with a matched placebo in individuals with COPD who require LTOT and use AOT during exercise. All participants provided informed consent. The study was registered prospectively in a publicly accessible database. The data presented here relate only to the planned COPD cohort in that study.

People with Global Initiative for Chronic Obstructive Lung Disease grade II–IV COPD who were established users of LTOT, in accordance with the NICE guidelines, were recruited from the outpatient clinical services of Royal Brompton and Harefield NHS Foundation Trust (North West London) between 4 November 2016 and 8 August 2017, with the last participant’s final visit completed on 15 January 2018.

Exclusion criteria for the study included clinical instability (ie, less than 1 month after an exacerbation), significant comorbidity limiting exercise tolerance, significant renal impairment (estimated glomerular filtration rate <50 mL/min), hypotension (systolic blood pressure < 100 mm Hg), pregnancy, use of NO$_3^-$-based medicine or phosphodiesterase V inhibitors, or presence of other conditions that might be influenced by NO$_3^-$ supplementation (ie, ischaemic heart disease or peripheral vascular disease). These conditions were assessed at the screening visit through review of clinical history and assessment of relevant clinical data.

Methods

Interventions

The intervention was a commercially available concentrated NO$_3^-$-rich BRJ (NR-BRJ) (98%) drink cut with organic lemon juice (2%) containing 0.8 g, 12.9 mmol NO$_3^-$ (140 mL Beet-It Sport Shot, James White Drinks, Ipswich, UK). The placebo beetroot juice (PL-BRJ), produced by James White, was 140 mL of the same beverage in which NO$_3^-$ was removed by a standardised method of passing the juice, prior to pasteurisation, through an ion exchange column, containing Purolite A520E, which exchanges NO$_3^-$ against chloride. The PL-BRJ is identical in appearance, packaging, taste and smell, and also causes beeturia (orange to red discoloration of urine).

Study conduct

At an initial baseline visit, COPD Assessment Test, Hospital Anxiety and Depression Scale, and Medical Research Council Dyspnoea Scale scores were recorded. Body composition was measured by bioelectrical impedance analysis using a Bodystat 4000 device (Bodystat, Isle of Man, UK). Participants then performed two incremental shuttle walk tests to determine the walking speed to be used for the ESWT and then a practice ESWT. All walking tests throughout the study were performed on the participant’s usual AOT flow rate, and the method for carrying the AOT was recorded to ensure the same method was always used (online supplemental appendix figure E1).

Prior to the two subsequent intervention visits and throughout the study period, participants were asked to avoid the use of antimicrobial mouthwash and chewing gum, as these have been shown to reduce the oral facultative bacteria whose NO$_3^-$ reductase activity is essential for the metabolism of an oral NO$_3^-$ load. They were asked to consume the same meal on the morning of each study assessment. This was to create as standardised conditions as possible, reducing differing levels of dietary NO$_3^-$ consumption as a source of variation within individuals, while not altering their usual diet greatly. They were also asked to match caffeine consumption to standardise any ergogenic effect arising from it and to avoid strenuous exercise in the 24-hour period prior to the intervention visits.

The two intervention visits began at the same time of day (±2 hours), with a minimum of 7-day washout period and a maximum 1-month gap between them. Participants were randomly assigned to the order in which they received NR-BRJ or PL-BRJ using a computer-generated block randomisation list, with a block size of 10, produced by an independent statistician. The researchers responsible for enrolment and outcome measurements remained blinded throughout the study and during data analysis. Following their arrival, after a 10 min rest period, participants were observed consuming either the NR-BRJ or the PL-BRJ, and empty bottles were collected and recorded. All outcome measures were undertaken 3 hours after ingestion of either NR-BRJ or PL-BRJ.

Outcomes

Exercise capacity

The primary outcome was ESWT compared between treatment conditions. Given the cross-over design and taking 65 s (95% CI 45 to 85) to be the minimal clinically important difference (MCID) in ESWT and a pooled mean difference within individuals of 26 s for repeat testing, to have an 80% statistical power, with a significance level of 0.05, 16 participants would be required to reject the null hypothesis that the active intervention was not superior to placebo. To allow for a 25% withdrawal rate, a sample size of 20 was chosen.

Plasma nitrate/nitrite levels and markers of oxidative stress

Plasma NO$_3^-$ and NO$_2^-$ levels were used as a combined biomarker of NO$_3^-$ ingestion, metabolism and NO availability. Plasma samples were obtained on arrival and 3 hours after consumption of NR-BRJ or PL-BRJ (see online supplemental appendix for full details).

Oxidative stress biomarkers were assessed in plasma samples by a combination of three distinct readouts, including antioxidative potential, that is, measurement of the ferric-reducing ability of plasma (FRAP), lipid oxidation products by thiobarbituric acid reactive substances (TBARS) and total free thiols with normalisation for protein (see online supplemental appendix for full details).

Fractional exhaled nitric oxide

FE$_{NO}$ was measured as a steady exhalation rate of 50 mL/s with a NIOX Mino (Aerocine Systems, Solna, Sweden) at the screening visit and then at the intervention visits at baseline prior to NR-BRJ/PL-BRJ consumption and then at six further intervals (30, 60, 90, 120, 150 and 180 min). Both the study participant


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and the researchers were blinded to the results, and an independent researcher, not directly involved with the trial, uploaded the data into a password-protected database. These data were only available to the researchers following unblinding of the study.

**Endothelial function**
Endothelial function was assessed by flow-mediated dilatation (FMD) of the brachial artery 3 hours after NR-BRJ/PL-BRJ consumption using a high-resolution Doppler ultrasound to measure at baseline and sequentially over a period of 120 s after release of circulatory arrest of the upper arm. All measurements were performed by a single trained operator (see online supplemental appendix for full details).

**Continuous oxygen saturation and heart rate analysis**
For each ESWT performed, pulse oximetry values were recorded (Pulsox 300i Pulse Oximeter, Konica Minolta, Tokyo, Japan) throughout until the participant had recovered (recovery was defined by return of Borg Dyspnoea Scale to that recorded prior to the ESWT). To maintain blinding, the pulse oximeter display was covered throughout the testing and the data were downloaded by an independent researcher, not directly involved with the trial, who uploaded the data to a password-protected database. These data were only available to the researchers following unblinding of the trial.

**Statistical analysis**
Data are presented as mean (SD), or if not normally distributed as median and IQR. Differences in response between treatment conditions were assessed using a paired t-test or a Wilcoxon signed-rank test as appropriate. Treatment effect was estimated using the Hodges-Lehmann estimate of shift parameters. The process of determining the Hodges-Lehmann estimator entails estimating the average difference in outcomes (x−y) for every possible n(n+1)/2 pair and then deriving the overall median of all averages (the Hodges-Lehmann estimator). A distribution-free CI is estimated using large-sample approximation. Analysis was performed using SPSS V.24 for Windows and Stata V.16.1 for Windows.

To compare continuous oxygen saturations (SpO2) and heart rate (HR) between the two treatment conditions, individual ESWT data periods were subjected to a 30 s rolling average using MATLAB (MATLAB and Statistics Toolbox Release V.2017a, The MathWorks, Natick, Massachusetts, USA) and then expressed as percentages of isotime (defined as the duration of the shortest of the two ESWTs). These individual responses were then grouped to allow analysis of HR and SpO2 against the percentage of isotime (plotted at the midpoint of each 10th percentile of isotime). The area under the curve (AUC) was assessed for each individual participant and the two treatment conditions compared using Wilcoxon signed-rank test. Figures were prepared using GraphPad Prism V.6.0 for Windows (GraphPad Software, San Diego, California, USA). A p value of <0.05 was considered statistically significant.

**RESULTS**
We screened 67 people for eligibility (figure 1); 31 declined to participate, 7 had a comorbidity precluding participation and 9 were not using supplementary oxygen. Of the 20 participants enrolled in the study, 10 were randomised to receive PL-BRJ first and 10 NR-BRJ first. All participants completed the study. Table 1 shows their baseline characteristics, which were well matched between the two order allocation groups. There were no serious adverse effects reported, although all participants reported beeturia. The average time between each intervention visit was 7 days.

**Exercise outcomes**
Exercise endurance time was longer for all study participants after NR-BRJ compared with PL-BRJ (figure 2): median (IQR) ESWT: NR-BRJ 194.6 (147.5–411.7) s vs PL-BRJ 159.1

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**Figure 1**  CONSORT diagram for recruitment and trial completion. CONSORT, Consolidated Standards of Reporting Trials; NR-BRJ, nitrate-rich beetroot juice; PL-BRJ, placebo beetroot juice.
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There was no evidence of an intervention order effect (online supplemental appendix figure E2).

There was one individual who was a clear outlier for exercise endurance response. However, a sensitivity analysis removing their data led to a slight change in primary study outcome but not the overall statistical significance: median (IQR) ESWT: NR-BRJ 193.8 (145–389.6) s vs PL-BRJ 158.2 (121.6–236.6) s, estimated treatment effect 56.5 (95% CI 30 to 88) s, indicating a significant increase in ESWT with NR-BRJ (p=0.0001) (online supplemental appendix results E1).

Pulse oximetry data were available for only 18 participants because recording failed for 2 of them. The average AUC for SpO2 was higher in the NR-BRJ group compared with the PL-BRJ group. These differences were more apparent at isotime and peak exercise, with no difference at rest, during warm-up or recovery (figure 3A and online supplemental appendix table E1). The estimated treatment effect was also statistically significant: 43.69 (29.09–58.28) (p<0.0001). The AUC for HR response to NR-BRJ or PL-BRJ did not show any difference. The estimated treatment effect was also not statistically significant (−41.17 (−116.74 to 34.40), p=0.27) (figure 3B and online supplemental appendix table E1).

Endothelial function and blood pressure

Two participants declined the FMD assessment; therefore, data were available for 18 participants. The average AUC for FEV1 was higher in the NR-BRJ group compared with the PL-BRJ group. These differences were more apparent at isotime and peak exercise, with no difference at rest, during warm-up or recovery (figure 3A and online supplemental appendix table E1). The estimated treatment effect was also statistically significant: 43.69 (29.09–58.28) (p<0.0001). The AUC for HR response to NR-BRJ or PL-BRJ did not show any difference. The estimated treatment effect was also not statistically significant (−41.17 (−116.74 to 34.40), p=0.27) (figure 3B and online supplemental appendix table E1).

Table 1  Characteristics of cross-over allocation groups: NR-BRJ or PL-BRJ received first

<table>
<thead>
<tr>
<th>Measurement</th>
<th>NR-BRJ first (n=10)</th>
<th>PL-BRJ first (n=10)</th>
<th>P value</th>
<th>Whole group (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% female: male)</td>
<td>30:70</td>
<td>50:50</td>
<td>1.0</td>
<td>40:60</td>
</tr>
<tr>
<td>Age (years)</td>
<td>68 (62–73)</td>
<td>67 (64–76)</td>
<td>0.7</td>
<td>67.6 (8.5)</td>
</tr>
<tr>
<td>Caucasian (%)</td>
<td>100</td>
<td>100</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Smoking (pack years)</td>
<td>28 (14–50)</td>
<td>64 (57–96)</td>
<td>0.006</td>
<td>52 (21.6)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 (20.9–29.0)</td>
<td>26.2 (21.4–30.1)</td>
<td>0.5</td>
<td>25.2 (4.7)</td>
</tr>
<tr>
<td>FFMI (kg/m²)</td>
<td>18.4 (15.8–20.1)</td>
<td>18.0 (14.0–20.2)</td>
<td>0.8</td>
<td>18.1 (15.8–19.9)</td>
</tr>
<tr>
<td>Inhaled medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SABA (%)</td>
<td>91</td>
<td>100</td>
<td>0.4</td>
<td>95</td>
</tr>
<tr>
<td>LABA-ICS (%)</td>
<td>91</td>
<td>78</td>
<td>0.4</td>
<td>95</td>
</tr>
<tr>
<td>LAMA (%)</td>
<td>91</td>
<td>100</td>
<td>0.4</td>
<td>85</td>
</tr>
<tr>
<td>Baseline resting oxygen saturation FiO2 0.21 (%)</td>
<td>92 (89–94)</td>
<td>92 (89–93)</td>
<td>0.8</td>
<td>92</td>
</tr>
<tr>
<td>Pre-LTOT prescription baseline pO2 (kPa)</td>
<td>6.9 (6.0–7.3)</td>
<td>6.6 (6.4–7.2)</td>
<td>0.6</td>
<td>6.8</td>
</tr>
<tr>
<td>Oxygen prescription (L/min)</td>
<td>4 (2–6)</td>
<td>2 (2–4)</td>
<td>0.2</td>
<td>3.0 (2.0–6.0)</td>
</tr>
<tr>
<td>CAT score</td>
<td>20 (18–29)</td>
<td>19 (15–28)</td>
<td>0.6</td>
<td>21 (8.0)</td>
</tr>
<tr>
<td>MRC Dyspnoea Score</td>
<td>4 (4–4)</td>
<td>4 (4–4)</td>
<td>1.0</td>
<td>4 (4–4)</td>
</tr>
<tr>
<td>HADS-A score</td>
<td>4 (3–7)</td>
<td>7 (2–10)</td>
<td>0.3</td>
<td>4.0 (2.3–8.8)</td>
</tr>
<tr>
<td>HADS-D score</td>
<td>4 (4–5)</td>
<td>5 (4–7)</td>
<td>0.7</td>
<td>4.5 (4.0–5.6)</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>139 (123–149)</td>
<td>135 (115–139)</td>
<td>0.1</td>
<td>137 (121–143)</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>76 (66–83)</td>
<td>70 (65–80)</td>
<td>0.4</td>
<td>73 (65–82)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>94 (91–104)</td>
<td>91 (85–94)</td>
<td>0.2</td>
<td>92 (80–100)</td>
</tr>
<tr>
<td>Lung function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1 (L)</td>
<td>0.7 (0.6–1.0)</td>
<td>0.7 (0.3–1.0)</td>
<td>0.3</td>
<td>0.7 (0.6–1.0)</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>2.7 (1.9–3.1)</td>
<td>1.6 (1.4–3.2)</td>
<td>0.3</td>
<td>2.7 (1.6–3.1)</td>
</tr>
<tr>
<td>FEV1:FVC ratio</td>
<td>0.3 (0.3–0.3)</td>
<td>0.3 (0.2–0.4)</td>
<td>1.0</td>
<td>0.3 (0.3–0.3)</td>
</tr>
<tr>
<td>RV %predicted</td>
<td>211 (181–235)</td>
<td>212 (188–233)</td>
<td>0.8</td>
<td>212 (186–233)</td>
</tr>
<tr>
<td>TLCO %predicted</td>
<td>33 (19–45)</td>
<td>36 (28–44)</td>
<td>0.9</td>
<td>32 (19–44)</td>
</tr>
<tr>
<td>GOLD stage</td>
<td>22</td>
<td>45</td>
<td>1.0</td>
<td>35</td>
</tr>
<tr>
<td>III (%)</td>
<td>78</td>
<td>55</td>
<td>1.0</td>
<td>65</td>
</tr>
<tr>
<td>ISWT distance (m)</td>
<td>300 (280–360)</td>
<td>370 (220–280)</td>
<td>0.04</td>
<td>279 (70)</td>
</tr>
<tr>
<td>ESWT (s)</td>
<td>172 (137–267)</td>
<td>181 (158–193)</td>
<td>0.6</td>
<td>179 (152–193)</td>
</tr>
</tbody>
</table>

Data in order of intervention, either NR-BRJ or PL-BRJ first.
Data shown are median (IQR), mean (SD) or percentage (%).
P value is for independent t-test or Mann-Whitney test comparing groups.
BML, body mass index; BP, blood pressure; CAT, COPD Assessment Test; ESWT, endurance shuttle walk test; FFMI, fat-free mass index; FiO2, fraction of inspired oxygen; GOLD, Global Initiative for Chronic Obstructive Lung Disease; HADS-A, Hospital Anxiety Depression Scale-Anxiety; HADS-D, Hospital Anxiety Depression Scale-Depression; ICS, inhaled corticosteroid; ISWT, incremental shuttle walk test; LABA, long-acting beta agonist; LAMA, long-acting muscarinic agonist; LTOT, long-term oxygen therapy; MAP, mean arterial pressure; MRC, Medical Research Council; NR-BRJ, nitrate-rich beetroot juice; PL-BRJ, placebo beetroot juice; RV, residual volume; SABA, short-acting beta agonist; TLCO, transfer Factor for carbon monoxide corrected for haemoglobin.

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**Figure 2** Effect of dietary nitrate supplementation on ESWT (in seconds) for PL-BRJ and NR-BRJ dosing conditions. Data presented as individual ESWT (in seconds) in both dosing conditions. Wilcoxon signed-rank test was used to compare ESWT between the different dosing conditions: NR-BRJ 194.6 (147.5–411.7) s vs PL-BRJ 159.1 (121.9–298.5) s, estimated treatment effect 62 s (95% CI 33 to 106). ***p<0.0001. ESWT, endurance shuttle walk test; NR-BRJ, nitrate-rich beetroot juice; PL-BRJ, placebo beetroot juice.

FMD increased: NR-BRJ 4.1% (−1.1% to 14.8%) compared with placebo −5.0% (−10.6% to −0.6%), estimated treatment effect −11.9% (95% CI −18.9 to −7.15) (p=0.0003). There was no statistically significant difference in change in blood pressure parameters from predosing levels between NR-BRJ and PL-BRJ (figure 3): median (IQR) ∆systolicBP: NR-BRJ −1.5 (15.0–10.8) mm Hg vs PL-BRJ −0.5 (−10.5 to 6.8) mm Hg, estimated treatment effect 1 mm Hg (95% CI −5.5 to 7.0) (p=1.0); ∆diastolicBP: NR-BRJ −4.0 (−14.0 to 7.0) mm Hg vs PL-BRJ −1.0 (−9.3 to 5.0) mm Hg, estimated treatment effect 1 mm Hg (95% CI −3 to 5) (p=0.481); mean arterial pressure: NR-BRJ −5.0 (−15.3 to 6.0) mm Hg vs PL-BRJ −2.5 (−13.5 to 7) mm Hg, estimated treatment effect 1.5 mm Hg (95% CI −3.5 to 5) (p=0.359).

**Plasma nitrate and nitrite levels and oxidative stress markers**

Paired data on plasma NO$_3^−$ and NO$_2^−$ concentrations were available for 19 participants, as 1 individual declined sampling. Following supplementation with NR-BRJ, there was an 84% increase in plasma NO$_3^−$ and an 887% increase in plasma NO$_2^−$ at 180 min post supplementation, but no change with placebo (figure 6 and online supplemental appendix table E2). Both the NR-BRJ and PL-BRJ supplements were analysed for NO$_3^−$ and NO$_2^−$ content as well (online supplemental appendix table E3). The change in plasma NO$_3^−$ and NO$_2^−$ from baseline to 180 s was calculated and used to estimate the treatment effect of NR-BRJ. The treatment effect of NO$_3^−$ was 550 (461–639) μM. The results suggest that this was higher for NR-BRJ than for PL-BRJ and this change was statistically significant (p=0.0003). The treatment effect of NO$_2^−$ was 0.248 (0.138–0.408) μM. The results suggest that this was higher for NR-BRJ than for PL-BRJ and this change was statistically significant (p=0.0011).

There was no statistically significant difference in measures of oxidative stress following acute consumption of either supplement: FRAP: NR-BRJ 1018 (853.0–1125) μM vs PL-BRJ 930.2 (836.8–1073) μM (p=1.0); TBARS: NR-BRJ 1.499 (0.855–3.209) mM vs PL-BRJ 0.971 (0.766–1.614) mM (p=0.4); total free thiol per protein: NR-BRJ 7.079 (5.961–8.115) μmol/g protein vs PL-BRJ 6.942 (5.768–8.026) μmol/g protein (p=0.5) (online supplemental appendix figure E3). Paired measures of FENO$_3$ were available for 16 participants at all seven time points (figure 7 and online supplemental appendix...
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Table E4). For four participants, there was device failure resulting in no data being recorded. The median (IQR) AUC for when the subjects were on PL-BRJ was 3622.5 (3181.9–4796.9) and the corresponding result for when the subjects were on NR-BRJ was 9440.6 (6273.8–11,831.3), and the treatment effect with its 95% CI was 5407 (3096 to 7576) (p=0.0011). The results suggest that the FENO levels while the subjects were on NR-BRJ were significantly higher than when they were on PL-BRJ.

Figure 4 Effect of dietary nitrate supplementation on blood pressure parameters. Change in blood pressure parameters (sBP, dBP and MAP) relative to baseline blood pressure 3 hours prior to dosing with either NR-BRJ or PL-BRJ. Data presented as 25th–75th percentile with the solid line representing the median value and the whiskers the minimum to maximum values. Wilcoxon signed-rank test was used to compare blood pressure parameters. Median (IQR) change in sBP: NR-BRJ −1.5 (15.0–10.8) mm Hg vs PL-BRJ −0.5 (−10.5 to 6.8) mm Hg (p=1.0). Median (IQR) in dBP: NR-BRJ 4.0 (−14.0 to 7.0) mm Hg vs PL-BRJ −1.0 (−9.3 to 5.0) mm Hg (p=0.481). Median (IQR) change in MAP: NR-BRJ −5.0 (−15.3 to 6) mm Hg vs PL-BRJ −2.5 (−13.5 to 7) mm Hg (p=0.359). dBP, diastolic blood pressure; MAP, mean arterial pressure; NR-BRJ, nitrate-rich beetroot juice; PL-BRJ, placebo beetroot juice; sBP, systolic blood pressure.

Figure 5 Effect of dietary nitrate supplementation on endothelial function. Percentage change in FMD from baseline and 180 min after supplementation with NR-BRJ or PL-BRJ. Data presented at the 25th and 75th percentile boxes with the solid line representing the median value and the whiskers the minimum and maximum values. Wilcoxon signed-rank test was used to compare the percentage change in FMD in the NR-BRJ (red) and PL-BRJ (black) dosing conditions. There was a statistically significant difference in the FMD percentage change with an increase in the NR-BRJ group (4.1, −1.1 to 14.8) versus a reduction in the PL-BRJ group (−5.0, −10.6 to −0.6). **p=0.0003. FMD, flow-mediated dilatation; NR-BRJ, nitrate-rich beetroot juice; PL-BRJ, placebo beetroot juice.

Figure 6 Plasma nitrite and nitrate levels. Data presented are median (IQR) with whiskers representing minimum to maximum values. Plasma NO₂⁻ and NO₃⁻ concentrations were measured at baseline (0 min) and 180 min after dosing with the interventions. Wilcoxon signed-rank test was used to compare change in plasma NO₂⁻ and NO₃⁻ concentrations between the intervention groups. Mann-Whitney U test was used to compare change in plasma NO₂⁻ and NO₃⁻ concentrations between the treatment conditions. (A) Changes in plasma NO₂⁻ concentrations. There was a statistically significant difference between baseline plasma NO₂⁻ concentration and postdosing with NR-BRJ for plasma NO₂⁻: predosing plasma NO₂⁻ concentration 0.306 (0.227–0.402) µM vs postdosing 0.620 (0.488–0.673) µM; ****p=0.000076. There was also a statistically significant difference between postdosing plasma NO₂⁻ concentration between NR-BRJ and PL-BRJ dosing conditions: postdose NR-BRJ NO₂⁻ concentration 0.620 (0.488–0.673) µM vs postdose of PL-BRJ NO₂⁻ concentration 0.306 (0.227–0.402) µM; †††† p=0.000009. (B) Changes in plasma NO₃⁻ levels. There was a statistically significant difference between baseline plasma NO₃⁻ concentration and postdosing with NR-BRJ for plasma NO₃⁻: predosing plasma NO₃⁻ concentration 62.59 (41.68–77.29) µM vs postdosing 617 (556.25–725.88) µM; ****p=0.00004. There was also a statistically significant difference between postdosing plasma NO₃⁻ concentration between NR-BRJ and PL-BRJ dosing conditions: postdose NR-BRJ NO₃⁻ concentration 617.71 (556.25–725.88) µM vs PL-BRJ plasma NO₃⁻ concentration 45.31 (31.39–58.84) µM; †††† p=5.66×10⁻¹¹). NO₂⁻, nitrite; NO₃⁻, nitrate; NR-BRJ, nitrate-rich beetroot juice; PL-BRJ, placebo beetroot juice. Its 95%CI was 5407 (3096 to 7576) (p=0.0011). The results suggest that the FE NO₂⁻ levels while the subjects were on NR-BRJ were significantly higher than when they were on PL-BRJ.

Postacute (0 min) supplementation with NR-BRJ FE\textsubscript{NO} increased by 184\% at 180 min post supplementation.

**DISCUSSION**

The major finding of this study was that, in people with COPD who are hypoxic to the extent that they meet the criteria for LTOT, dietary NO\textsuperscript{3−} supplementation improves exercise capacity compared with placebo. The improvement in ESWT that we observed was accompanied by less desaturation during exercise. Supplementation also improved endothelial function assessed using FMD. In line with previous observations, exercise and daily physical activity. This was a single-dose response or ceiling effect. We have also shown that the improvement in walking time was therefore encouraging, although further studies of longer-term use will be needed before any clinical recommendations can be made.

**Significance of findings**

Although studies have previously considered dietary NO\textsuperscript{3−} supplementation in COPD with inconsistent results, this is the first stratified medicine approach focusing on the specific phenotype of individuals with COPD with hypoxaemia requiring LTOT. Our previous study, in non-hypoxaemic patients with COPD, found that there was a reduction in the oxygen cost of exercise during cycle ergometry yet no improvement in exercise capacity. In conditions of hypoxia, the L-arginine–nitric oxide synthase pathway is compromised, while the NO\textsuperscript{−}–NO\textsuperscript{3−}–NO pathway is facilitated due to a lesser inhibition of NO\textsuperscript{−} biotransformation by oxygen. As such, dietary NO\textsuperscript{3−} supplementation could be expected to have more impact in hypoxic rather than normoxic individuals, both through effects on the skeletal muscle and impacts on the pulmonary vasculature.

The mechanism by which ESWT lengthened is likely to involve multiple synergistic pathways. The finding of relatively preserved Sp\textsubscript{O2} during exercise in the NO\textsuperscript{−}–supplemented condition could reflect more efficient oxygen utilisation peripherally, a beneficial impact on central haemodynamics associated with related to reduced hypoxia-induced pulmonary vasconstriction, or a combination of the two. Despite each participant using their prescribed oxygen for each walk test, there was an observed desaturation in the placebo arm. This could mean that their oxygen prescription may have been insufficient and that a higher flow rate might also have increased exercise capacity. This finding of the attenuation of desaturation by NR-BRJ may well be explained by the enhancement of the NO\textsuperscript{−}–NO\textsuperscript{3−}–NO pathway in conditions of hypoxia.

The observation that NO\textsuperscript{3−} supplementation was associated with improvements in endothelial function assessed using FMD is likely to be relevant to the acute mechanism of benefit from NO\textsuperscript{3−} supplementation, but also raises the possibility that longer-term dosing might reduce the risk of vascular events which are common in COPD. The effects seen are almost certainly not COPD-specific and work is needed to investigate possible benefits in other long-term lung conditions associated with hypoxia, including interstitial lung diseases and the various categories of pulmonary hypertension.

The estimated treatment effect of dietary NO\textsuperscript{3−} supplementation on ESWT found in this study was 62.5 s, which falls fractionally short of the MCID defined in pharmacotherapy trials as 65 s. However, in pharmacological trials where the ESWT is the outcome, interventions are typically administered over weeks or months. The demonstration of an effect of similar magnitude in a single-dose study is therefore encouraging, although further studies of longer-term use will be needed before any clinical recommendations can be made.

**Study limitations**

The use of a robust placebo strengthens the reliability of the findings, as does the fact that the improvement in walking time was accompanied by an appropriate physiological response (lower HR and higher Sp\textsubscript{O2}). An additional strength was the use of a walking rather than a cycling test, which is of clinical relevance to patients as it reflects most individuals’ main form of exercise and daily physical activity. This was a single-dose study and therefore questions remain as to the impact that regular dosing might have and whether this would translate into meaningful clinical effects. The dose used was selected based on previous studies, but future work should investigate whether there is a dose response or ceiling effect. We have also shown that the NR-BRJ does indeed contain a higher quantity of NO\textsuperscript{3−} and provide independent confirmation that NO\textsuperscript{3−} is only present at very low levels in the placebo juice used in our study.

**CONCLUSION**

BRJ is cheap and readily accessible and has the potential to be used widely as a dietary supplement if effective in specific patient groups. Its beneficial effects appear to be mediated by inorganic NO\textsuperscript{3−} without affecting plasma redox status, on acute administration. Further mechanistic work is needed to work out the relative impact of the possible mechanisms, in particular the impact of muscle versus pulmonary or cardiac/systemic circulation effects, and longer-term studies will be needed to establish if the effects on exercise performance and endothelial function observed here translate into clinically meaningful benefits.
Individual participant data that underlie the results in the article after declaration of Helsinki. P in the first draft of the manuscript. All authors edited and contributed to the final manuscript. NSH is the guarantor.

None declared.

Not required.

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Not required.

Data are available upon reasonable request. Individual participant data that underlie the results in the article after de-identification (text, tables, figures and appendices) will be made available from the corresponding author upon request. The study protocol and statistical analysis plan will also be available. Data will be available indefinitely.

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