CLINICAL STUDY PROTOCOL

(ICTU ADOPTED)

Full Study Title: Dietary nitrate supplementation to enhance daily physical activity in hypoxic COPD: a randomised controlled trial

Short Study title / Acronym: The ON-PACE trial

Sponsor: Imperial College London

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This protocol describes the ON-PACE trial and provides information about procedures for enrolling participants to the trial. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study, but centres enrolling participants for the first time are advised to contact the Trial Coordination centre to confirm they have the most recent version. Problems relating to this trial should be referred, in the first instance, to the Trial Coordination centre.

This trial will adhere to the principles outlined in the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act 2018 and other regulatory requirements as appropriate.

This study/project is funded by the NIHR Efficacy and Mechanism Evaluation (EME) Programme (NIHR131548). The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

ABBREVIATIONS

ADMA	Arginine/Asymmetric dimethylarginine
AE	Adverse Event
ATP	Adenosine Triphosphate
BRJ	Beetroot Juice
CI	Chief Investigator
COPD	Chronic obstructive pulmonary disease
CRF	Case Report Form
DMEC	Data Monitoring and Ethical Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ESWT	Endurance shuttle walk time
GCP	Good Clinical Practice
HRA	Health Research Authority
ICH	International Conference of Harmonisation
ICHNT	Imperial College Healthcare NHS Trust
ICMJE	International Committee of Medical Journal Editors
ICTU	Imperial Clinical Trials Unit
ISWT	Incremental Shuttle Walk Test
ITT	Intention to Treat
NHLI	National Heart and Lung Institute
NIHR	National Institute for Health Research
NOS	Nitric Oxide Synthase
NR-BRJ	Nitrate-rich Beetroot Juice
PL-BRJ	Placebo Beetroot Juice
PR	Pulmonary Rehabilitation
PROactive	Patient reported outcome for physical activity in COPD
QA	Quality Assurance
QC	Quality Control
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan

SOP	Standard Operating Procedure
TMG	Trial Management Group
TSC	Trial Steering Committee

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TRIAL SUMMARY

TITLE: Dietary nitrate supplementation to enhance daily physical activity in hypoxic COPD: a randomised controlled trial. The ON-PACE study.

OBJECTIVES

Primary objective: To establish if dietary nitrate supplementation reduces the difficulty that patients with hypoxic COPD experience with day-to-day physical activity.

Secondary objectives are whether there are associated improvements in exercise capacity, blood pressure, quality of life and endothelial function.

DESIGN A Phase III, double-blind, parallel group, randomised controlled trial.

SAMPLE SIZE 102

ELIGIBILITY

Inclusion:

1) Adults over 18 years of age
2) Diagnosis of COPD
3) Under care of home oxygen service
4) Documented oxygen assessment meeting NICE criteria (i) (PaO_2<7.3 or <8 if pulmonary hypertension) or (ii) increase in exercise capacity if on ambulatory oxygen only

(i) Exclusion criteria

1) Inability to consent
2) Systolic blood pressure below 120mmHg
3) Exacerbation within the last four weeks
4) Use of nitrate-based medication
5) Current participation in another clinical trial

INTERVENTION Participants will be randomly allocated to either:

- three months of dietary nitrate supplementation with beetroot juice (70mL Beet-It® SPORT shot; 6.4mmol nitrate, James White Ltd) once daily

or

- Three months of an otherwise identical placebo juice from which the nitrate has been removed (70mL nitrate deplete placebo equivalent shot, James White Ltd).

OUTCOME MEASURES

PRIMARY ENDPOINT

- Patient reported outcome - the difficulty domain of the clinic visit physical activity (PROactive COPD tool – c-PPAC)

SECONDARY ENDPOINTS

- Improved experience of amount of daily physical activity (PROactive COPD tool – c-PPAC)
- Exercise capacity - endurance shuttle walk time (ESWT)
- Oxygenation and lower heart rate during exercise
- Mean arterial pressure
- Step count/day
- Quality of life (CAT score)
- Fatigue (FACIT score)
- EQ-5D-5L
- BNP levels
- Endothelial function assessed using the Endopat device
- Blood markers of endothelial function (ADMA, Endothelin 1, Prostacyclin)
- Blood nitrite/nitrate levels

1. BACKGROUND

COPD is a common condition responsible for considerable morbidity and mortality. There are 1.3 million people with a diagnosis of COPD in the UK and existing treatments are relatively ineffective, meaning that many people with the condition are limited in their day to day activities(1) experiencing poor quality of life and social isolation. This is especially the case in those with “hypoxic COPD” the most severe group who require supplemental oxygen(2). Audit data suggest 0.4% of people with COPD, roughly 5,200 people in the UK, receive home oxygen. As the population ages this number is increasing. Multimorbidity, especially cardiovascular diseases are also extremely common in people with COPD.

There is considerable unmet need around the care of people with COPD and breathlessness(3, 4). Patients with hypoxic COPD have limited options – inhaled medication has only a modest effect on symptoms and although pulmonary rehabilitation is beneficial, patients remain limited and may have difficulty accessing it because of transport or other issues. There is a need to develop alternative strategies to improve exercise capacity and day to day physical activity in people with severe COPD.

People with COPD who require oxygen have severe disease, are extremely limited and have few therapeutic options available. There is a need for novel strategies to improve outcomes, especially in this patient phenotype. Activity limitation in COPD is dependent on ventilatory limitation and impaired gas exchange, cardiovascular function and skeletal muscle factors all of which are potentially influenced by nitric oxide signalling. Dietary nitrate supplementation augments the effect of pulmonary rehabilitation in COPD (ON-EPIC) (5) and the EDEN-OX trial showed that a single dose of dietary nitrate supplementation significantly increased exercise capacity in hypoxic individuals, but we do not know if this effect is sustained.

Beetroot juice (BRJ) drinks are commercially available, marketed for their potential health benefits and widely used by sports people. In patients with stable COPD that is sufficiently severe that they need to use supplemental oxygen, we hope to show that regular dietary nitrate supplementation in the form of beetroot juice will produce a sustained improvement in daily physical activity levels. As a relatively cheap, non-drug intervention, beetroot juice has the potential to be widely applicable and by preventing events ease the burden of ill health on the NHS and society. Increased physical activity, as well as translating into better quality of life for the thousands of hypoxic COPD patients affected, is also likely to reduce care needs and social isolation.

It is not at present known whether the improvement in exercise capacity that we have demonstrated following dietary nitrate supplementation in patients with a hypoxic COPD phenotype translates into meaningful clinical benefit.

We have undertaken a systematic review (PROSPERO 42019130123) to evaluate published studies to 1st July 2020 including (i) Adults diagnosed with chronic respiratory disease (ii) Nitrate supplementation as an intervention (iii) a control/placebo group for comparison looking at exercise capacity or cardiovascular parameters. After full-text review, 11 studies met the inclusion criteria; 2 RCTs and 9 cross-over studies. Apart from ON-EPIC, the largest study enrolled only 23 individuals. The range of different outcomes and patient populations limited possibilities for data synthesis.

Key findings are. (i) dietary nitrate supplementation reduces the oxygen cost of exercise in COPD(6) (ii) dietary nitrate supplementation increases exercise capacity in people with

COPD who are hypoxic (the EDEN-OX trial) (iii) dietary nitrate supplementation augments the effect of pulmonary rehabilitation on exercise capacity in people with COPD (the ON-EPIC trial)(5).
Other ongoing trials: A search for trials in COPD with “nitrate” or “beetroot” on https://apps.who.int/trialsearch/ [11-11-20] reveals no trials looking at physical activity levels or exercise capacity in hypoxic COPD.

Nitric oxide is an important signalling molecule with a wide range of effects including impacts on vascular function and skeletal muscle metabolism. In addition to the ‘classical’ oxidation of L-arginine catalysed by NO synthase (NOS) family of enzymes acting on the amino acid L-arginine, NO can be produced by the reduction of exogenous dietary nitrate (NO_3^-) in an oxygen independent manner (7), a complementary pathway allows for the generation of NO, the nitrate-nitrite-NO pathway. This nitrate-nitrite-NO pathway can be augmented via a dietary bolus of nitrate-rich food, including beetroot juice. This pathway is more active in hypoxic situations as nitrite is reduced to NO by deoxy-haemoglobin and –myoglobin and H^+ ions. Nitrate supplementation is thus, in effect, *a targeted therapy to hypoxic tissues.*

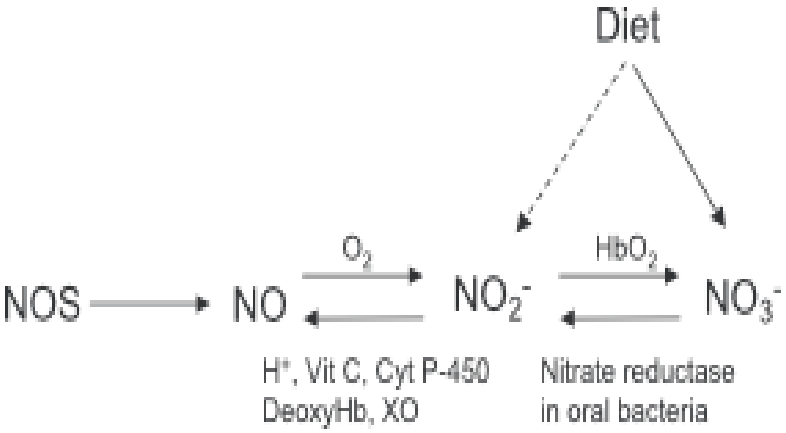


Figure 1. Nitrate-nitrite-NO pathway in humans. Plasma nitrate originates from the oxidation of endogenously produced NO and dietary sources. Nitrite reduction to NO is enhanced in acidic (H^+), hypoxic and reducing (e.g. vitamin C) conditions. Other proteins and enzymes may promote this step including Cytochrome P-450 (Cyt P-450), Xanthine Oxidase (XO) and Deoxyhaemoglobin (DeoxyHb). Taken from Lundberg et al.(8)

Research, initially in sports medicine, demonstrated a reduction in the oxygen cost of exercise following NO_3^- supplementation in healthy individuals (9) associated with improved exercise performance (9-11). Beetroot juice reduced the oxygen cost of all exercise modalities and extended the time to exhaustion during severe-intensity running by 15% (from 7.6 to 8.7 minutes).

It is likely that there are multiple NO related mechanisms contributing to the observed increase in exercise capacity following dietary NO_3^- supplementation including increased skeletal muscle oxygen delivery through enhanced blood flow to muscle, reduced adenosine triphosphate (ATP) cost to generate a submaximal force , increased mitochondrial efficiency by reducing the oxygen cost of mitochondrial ATP resynthesis and improved calcium handling in fast-twitch muscle fibres resulting in increased contractile force (12). Mitochondrial mechanisms are important in COPD given known mitochondrial dysfunction in COPD (13). In addition, effects on systemic and pulmonary circulation may improve cardiovascular function.

NO_3^- supplementation has also been shown to improve symptoms and walking distance in people with peripheral arterial disease, with lower gastrocnemius fractional oxygen extraction indicating improved metabolic efficiency (14).

We have now shown that dietary nitrate supplementation in the form of beetroot juice, has a number of potentially advantageous effects in COPD, providing proof of concept for this proposal. These include *reducing* the oxygen cost of exercise in people with less severe COPD (6) and *improving* exercise capacity in hypoxic COPD patients.

In a double blind cross over study in 20 hypoxic COPD patients (EDEN-OX), we found that a single dose of beetroot juice supplementation increased endurance shuttle walk time substantially compared to placebo(5), with an estimated treatment effect of 62sec (95%CI 33, 106) p<0.0001. This improvement in exercise capacity was accompanied by less desaturation and a lower heart rate during exercise. In this study active treatment also improved endothelial function assessed using measurement of brachial artery flow mediated dilatation.

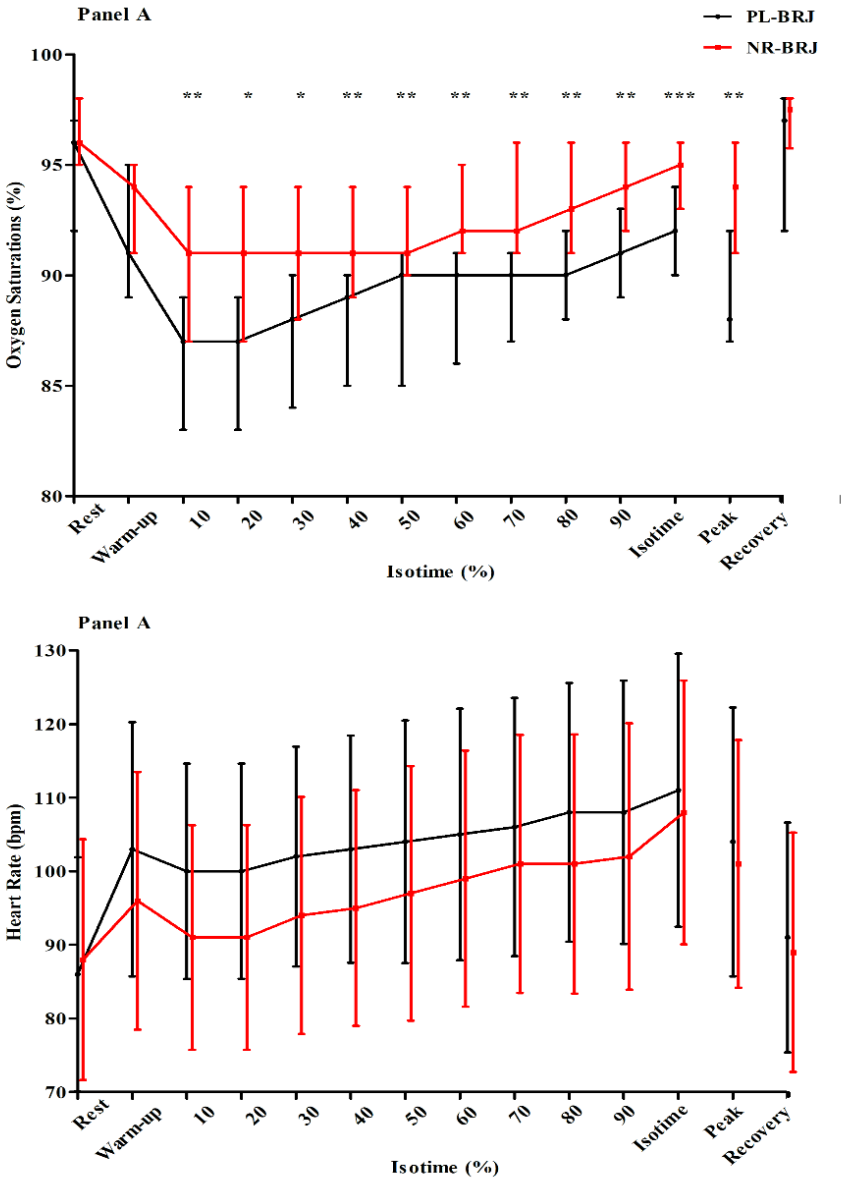


Figure 2: In the EDEN-OX trial cross over trial in hypoxic COPD patients using supplemental oxygen, nitrate supplementation was associated with lower heart rate and higher oxygen saturations to isotime in nitrate rich beetroot juice (NR-BRJ) compared to nitrate deplete placebo juice (PL-BRJ) during an endurance shuttle walk test, with an estimated treatment effect of 62sec (95%CI 33, 106) p<0.0001 increase in endurance walking time

We have also demonstrated that dietary nitrate supplementation *enhances* the gains in exercise capacity seen with pulmonary rehabilitation (PR) (the ON-EPIC trial). In this randomised controlled trial in 122 patients with stable COPD undergoing PR, exercise capacity increased more with active treatment than placebo; median (IQR) change in ISWT distance +60(10, 85)m vs. +30(0, 70)m, (p = 0.027).

Dietary nitrate supplementation also impacted on systolic blood pressure, with a reduction of 5.0 mmHg (-5.0, -3.0) vs an increase of 6.0 mmHg (-1.0, 15.5) ($p<0.0005$). No significant serious adverse events or side effects were reported over the course of 8 weeks of treatment.

Multimorbidity is the norm rather than the exception in COPD; 50% of COPD patients have three or more other long term conditions and at least 40% have concomitant cardiovascular disease(15). Dietary nitrate supplementation has been shown to reduce blood pressure both in normotensive (16) and hypertensive(17) subjects, though studies to date have been of relatively short duration – the longest for 5 weeks (reviewed here (18, 19)). There are also data linking NO with the development of metabolic syndrome - a clinical and biochemical expression of insulin resistance, representing a clustering of central obesity, hypertension, hyperglycaemia, and dyslipidaemia (20, 21).

Dietary nitrate supplementation in the form of beetroot juice has the potential both to improve exercise capacity, especially in hypoxic individuals, and thus make the experience of daily physical activity less difficult for people with COPD who require supplemental oxygen. There is also the potential that by improving endothelial function, the risk of vascular events can be reduced.

1.1 Clinical setting

The study will take place in secondary care recruiting people with COPD who are under the care of home oxygen services.

1.2 Intervention details

Patients will be randomised to either beetroot juice or nitrate-depleted placebo beetroot juice for 3 months.

The intervention is a commercially available concentrated NO_3^- rich beetroot juice (NR-BRJ) (98%) drink cut with organic lemon juice (2%), containing 0.4 g, 6.4 mmol of NO_3^- (70mL Beet-It® SPORT shot, James White Drinks, Ipswich, UK). The placebo is an identical beverage from which the nitrate has been removed.

1.3 Rationale for the study

As outlined above, dietary nitrate supplementation increases exercise capacity in people with COPD who are hypoxic. The ON-PACE study will evaluate whether this improvement translates into an improved experience of the difficulty of daily physical activity. It will also evaluate markers of endothelial function, improvement in which may reduce the risk of acute vascular events in this population.

1.4 Risk / Benefit Assessment

The intervention is a widely available nutritional supplement which has not been associated with any major side effects. Urine discolouration (beeturia) is common and gastrointestinal disturbance is reported by some individuals. Individuals in the active arm may benefit from the intervention if the trial hypothesis is found to be correct, and a positive result will provide a new intervention to improve quality of life in people with severe COPD. The study is therefore low risk, with a favourable risk / benefit ratio.

2. OBJECTIVES AND ENDPOINTS

2.1 Primary Objective

To establish the effect of dietary nitrate supplementation on experience of difficulty with physical activity.

2.2 Secondary Objective

To establish the effect of dietary nitrate supplementation on the experience of amount of daily physical activity, on objective physical activity levels, on quality of life, on exercise capacity as well as physiological response to exercise, and on physiological and blood markers of endothelial function.

2.3 Primary Endpoint

Patient reported outcome - the difficulty domain of the clinic visit physical activity (PROactive COPD tool – c-PPAC)

2.4 Secondary Endpoints

- Improved experience of amount of daily physical activity (PROactive COPD tool – c-PPAC)
- Exercise capacity - endurance shuttle walk time (ESWT)
- Oxygenation and heart rate during exercise
- Mean arterial pressure
- Step count/day
- Quality of life (CAT score, EQ-5D-5L
- Fatigue (FACIT score)
- BNP levels
- Endothelial function assessed using the Endopat device.
- Blood markers of endothelial function (ADMA, Endothelin 1, Prostacyclin)
- Blood nitrite/nitrate levels.

2.5 Summary Table of Objectives and Endpoints

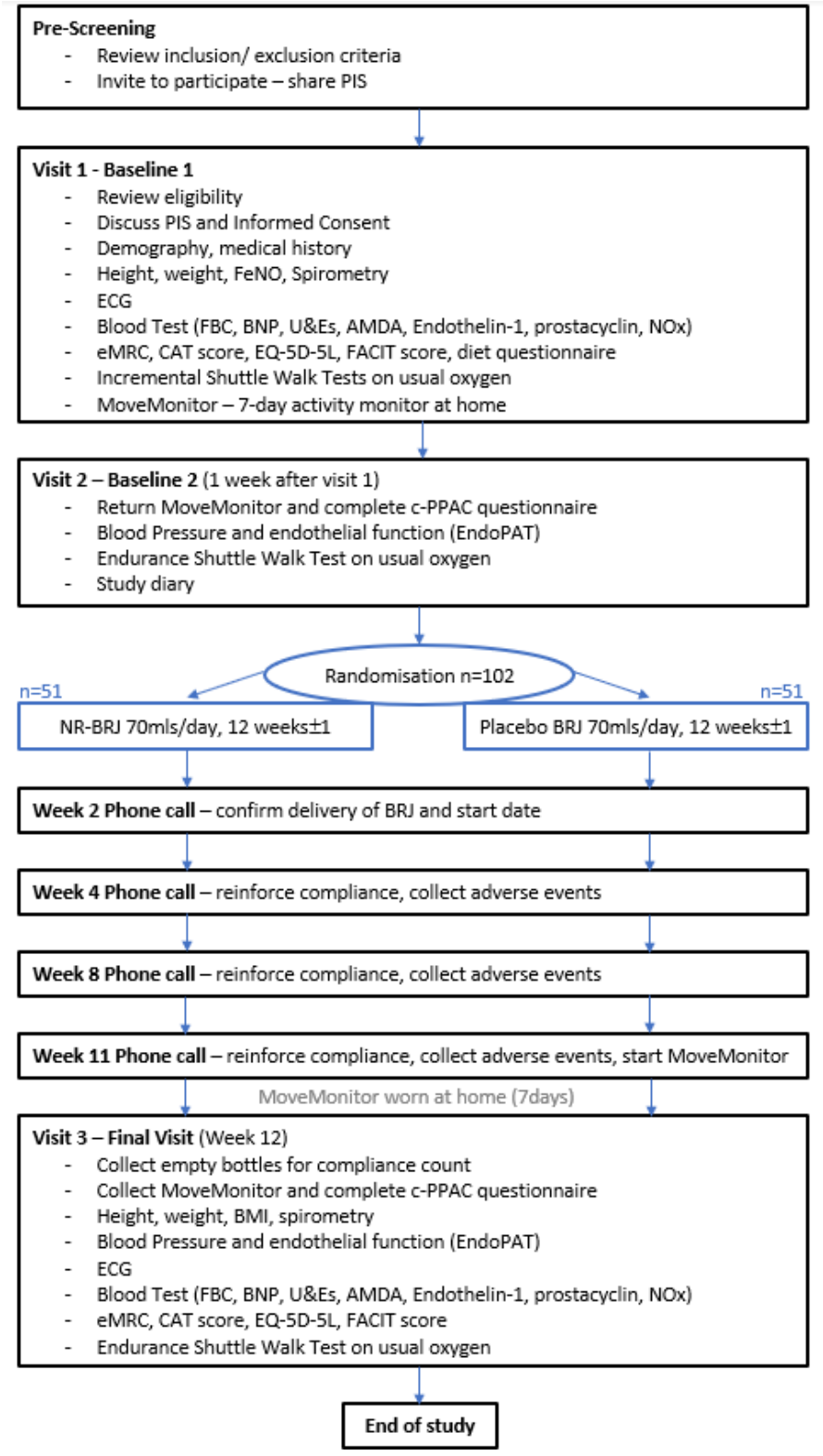
Objectives	Endpoints	Timepoint(s) of evaluation of this endpoint (if applicable)
Primary Objective To establish effect of dietary nitrate supplementation on experience of difficulty with physical activity	The difficulty domain of the clinic visit physical activity (PROactive COPD tool – c-PPAC)	Baseline and three months
Secondary Objectives To establish the effect of dietary nitrate supplementation on the experience of amount of daily physical activity, on objective physical activity levels, on quality of life, on exercise capacity as well as physiological response to exercise, and on physiological and blood markers of endothelial function.	Improved experience of amount of daily physical activity (PROactive COPD tool – c-PPAC) Exercise capacity - endurance shuttle walk time (ESWT) Oxygenation and heart rate during exercise Mean arterial pressure Step count/day Quality of life (CAT score/EQ-5D-5L) Fatigue (FACIT score) BNP levels Endothelial function assessed using the Endopat device. Blood markers of endothelial function (ADMA, Endothelin 1, Prostacyclin) Blood nitrite/nitrate levels.	Baseline and three months

3. STUDY DESIGN

3.1 Design

On-PACE is a double-blind parallel group randomised controlled trial to demonstrate the superiority of dietary nitrate supplementation with beetroot juice to matched placebo conducted across three sites, the Royal Brompton Hospital Clinical Research Facility, Glenfield Hospital, part of University Hospitals of Leicester NHS Trust, and Royal Sussex County Hospital, part of University Hospitals Sussex NHS Foundation Trust. There are also 4 patient identification sites to aid identification in London; 1. Guys and St Thomas (GSTT), 2. King's College (KCHFT), Imperial College (ICHT), and 4. Chelsea and Westminster Hospital NHS Foundation Trust.

Study Flow Chart


Pre-Screening
- Review inclusion/ exclusion criteria
- Invite to participate – share PIS
Visit 1 - Baseline 1
- Review eligibility
- Discuss PIS and Informed Consent
- Demography, medical history
- Height, weight, FeNO, Spirometry
- ECG
- Blood Test (FBC, BNP, U&Es, AMDA, Endothelin-1, prostacyclin, NOx)
- eMRC, CAT score, EQ-5D-5L, FACIT score, diet questionnaire
- Incremental Shuttle Walk Tests on usual oxygen
- MoveMonitor – 7-day activity monitor at home
Visit 2 – Baseline 2 (1 week after visit 1)
- Return MoveMonitor and complete c-PPAC questionnaire
- Blood Pressure and endothelial function (EndoPAT)
- Endurance Shuttle Walk Test on usual oxygen
- Study diary
Randomisation n=102
n=51
n=51
NR-BRJ 70mls/day, 12 weeks±1
Placebo BRJ 70mls/day, 12 weeks±1
Week 2 Phone call – confirm delivery of BRJ and start date
Week 4 Phone call – reinforce compliance, collect adverse events
Week 8 Phone call – reinforce compliance, collect adverse events
Week 11 Phone call – reinforce compliance, collect adverse events, start MoveMonitor
MoveMonitor worn at home (7days)
Visit 3 – Final Visit (Week 12)
- Collect empty bottles for compliance count
- Collect MoveMonitor and complete c-PPAC questionnaire
- Height, weight, BMI, spirometry
- Blood Pressure and endothelial function (EndoPAT)
- ECG
- Blood Test (FBC, BNP, U&Es, AMDA, Endothelin-1, prostacyclin, NOx)
- eMRC, CAT score, EQ-5D-5L, FACIT score
- Endurance Shuttle Walk Test on usual oxygen
End of study

4. PARTICIPANT ENTRY

4.1 Study setting and population

(i) Inclusion criteria

Stable, adult patients with COPD who are hypoxic and are users of home oxygen - ambulatory alone, or in combination with long term oxygen therapy, prescribed and documented in accordance with NICE guidance for COPD (22). It is important to note that recruitment will be occurring within the context of well-established oxygen services. Patients will only be enrolled if there is a clear record that appropriate assessment has been carried out to justify oxygen prescription including, in people on ambulatory oxygen only, a formal ambulatory oxygen assessment demonstrating an improvement in walk distance.

1) Adults over 18 years of age
2) Diagnosis of COPD
3) Under care of home oxygen service
4) Documented oxygen assessment meeting NICE criteria (i) (PaO_2<7.3 or <8 if pulmonary hypertension) or (ii) increase in exercise capacity if ambulatory oxygen only

(ii) Exclusion criteria

1) Inability to consent
2) Systolic blood pressure below 120mmHg
3) Exacerbation within the last four weeks
4) Use of nitrate-based medication

5. PROCEDURES AND MEASUREMENTS

5.1 Identification and recruitment of participants

Participants will be recruited from home oxygen services across London (including those based at Royal Brompton Hospital, Guys and St Thomas, Kings, and Imperial College Healthcare), Leicester and Brighton. Recruitment will be by

(i) An invitation letter/information sheet will be sent to potentially eligible people identified by the responsible clinical team using their clinical database.
(ii) opportunistic identification when patients attend for clinical follow up.
(iii) the study will be advertised through local BreatheEasy Groups with an online link where potential participant can review eligibility criteria and register interest.
(iv) NIHR Be Part of Research mailout

We will establish an online form where potential participants can register their interest and go through the basic eligibility questions. With patient agreement their oxygen records will be sent to the research team by their home oxygen clinical team so that eligibility can be confirmed.

5.2 Screening and pre-randomisation evaluations

Written informed consent will be obtained before the participant undergoes any screening procedures. Having reviewed consent and eligibility baseline assessments will be carried out in two visits.

5.3 Randomisation and Blinding

Randomisation and unblinding will be carried out using a web-based randomisation and Electronic Data Capture (EDC) system, called OpenClinica. Trial data will also be collected on OpenClinica EDC.

This will be programmed with a variable block randomisation schedule, stratified by oxygen use (long-term oxygen vs only ambulatory oxygen) and eMRC dyspnoea score into two groups ($\leq$4 vs >4).

Participants will receive blinded, identical-looking and identically packaged trial intervention of either beetroot juice or placebo control.

Participants will be identified with a unique trial identifier and each beetroot juice or placebo drink will be identified with a unique treatment code linked to the allocation and trial identification (ID).

5.4 Code-breaking/ Unblinding

Each participant will be assigned a unique trial ID and each bottle dispensed will be identified with a unique treatment code which is linked to the treatment allocation. The treatment code must not be broken except in medical emergencies when the appropriate management of the participant necessitates knowledge of the treatment, or in the event that expedited reporting to the Research Ethics Committee (REC) of an unexpected and related Serious Adverse Event (SAE) is required.

The trial EDC system will include an automated unblinding facility, in case unblinding is required. In the event that emergency unblinding of an individual participant is required, authorised staff (as documented on the delegation log) will follow trial procedures to unblind the participant in question and proceed with expedited reporting if required.

Unblinding should only be considered if management of the participant would differ depending on whether they are on beetroot juice or control.

5.5 Visit Schedule

Visit 1 (Screening/Baseline). Review eligibility and informed consent obtained. Baseline measures include an ECG, CAT score, FACIT score, EQ-5D-5L, MAP, blood pressure spirometry and FeNO measurement as well as height and weight - BMI. eMRC dyspnoea score. Diet questionnaire will be administered. Blood pressure will be measured.

Blood drawn for FBC, U&Es, BNP, Endothelin 1, prostacyclin, nitrate/nitrite levels and Arginine/Asymmetric dimethylarginine (ADMA).

Participants perform two incremental shuttle walk tests - the better result of these is used to determine the ESWT speed to be used. They will be given a McRoberts MoveMonitor to wear for one week on their waist.

Visit 2 (Baseline 1) one week later. Participants return the MoveMonitor and complete the PROactive physical activity (c-PPAC) questionnaire.

Endothelial function assessed using the Endopat device.

ESWT performed on the participant's usual ambulatory oxygen flow rate. A diary will be given to the patient to complete.

Random allocation to study arm.

Trial intervention delivered to their home and participants start to take daily active/placebo beetroot juice.

2-week phone call. Used to confirm BRJ has been delivered to participant home. Start date of BRJ consumption is also recorded.

4-week phone call. Used to reinforce compliance/collect information about any adverse events. It will also be used to check the delivery of the study intervention (beetroot juice/placebo)

8-week phone call. Used to reinforce compliance/collect information about any adverse events.

11-week phone call. MoveMonitor posted to participants in week 10. Phone call to remind participants to wear the monitor for a week up till the final study visit.

Visit 3 week 12 Participants return for end of study assessment.

We will collect empty bottles for compliance count and ask directly about compliance and adverse events. Diet questionnaire.

Participants will have an ECG, complete the CAT score and MRC dyspnoea score and have their weight measured. Repeat baseline blood tests. Adverse events will be collected.

Participants will return the MoveMonitor to allow analysis of physical activity data and complete the PROactive physical activity (c-PPAC) questionnaire. After a rest period the EndoPat measure of endothelial function will be performed as well as checking blood pressure.

They will then perform an ESWT on their usual oxygen and exit the trial.

Summary of visit activities

Assessment	Pre-screen	Visit 1 Baseline Wk0	Visit 2 Baseline Wk1	Wk2	Wk4	Wk8	Wk10	W11	Visit 3 Final Wk12
PIC Patient identification	X								
Informed consent		X							
Inclusion & exclusion criteria		X							
Demography		X							
Medical history		X							
eMRC		X							X
ECG		X							X
Spirometry		X							
FeNO		X							
Height/weight/BMI		X							
CAT score		X							X
FACIT score		X							X
EQ-5D-5L		X							X
Diet questionnaire		X							
Blood draw (FBC, U&Es, BNP, ADMA, Endothelin 1, Prostacyclin, NOx)		X							X
Endothelial function (EndoPAT)			X						X
BP		X							X
ISWT		X							
Given MoveMonitor		X							
Collect MoveMonitor			X						X
c-PPAC			X						X
ESWT			X						X
Randomisation			X						
Given diary/collect diary			X						X
Phone call- check delivery of BRJ				X					

Phone call to check trial compliance					X	X		X	
Adverse events					X	X	X	X	X
MoveMonitor posted out							X		
Instruct to wear MoveMonitor								X	
Collect empty bottles									X

5.6 Study measurements

Patient experience of physical activity, which is the primary endpoint of the study, will be assessed using the *c*linic visit *P*ROactive consortium *P*hysical *A*ctivity in *C*OPD (c-PPAC) tool. This was developed as part of the EU/IMI PROactive consortium which included the applicants. The tool combines activity monitoring data and questionnaire responses. This tool, the primary endpoint for the study, although recently developed is a well-validated measure (23-26). The creation of this tool proceeded in accordance with FDA and EMA manuals for the development of patient reported outcome (PRO) measures. This five-year process allowed the production of a patient reported outcome measure that could be used in clinical trials and to support labelling claims around physical activity. This required the construction of a valid conceptual framework for physical activity, qualitative and quantitative work in a large multinational patient cohort, and an item reduction process to make the tool as practical as possible while retaining its discriminant validity.

The concept of patient experience of physical activity in COPD was found through the development process to contain two domains; “amount” and difficulty”. Difficulty is based on questionnaire responses alone, whereas amount is derived from a combination of questionnaire data (how individuals feel about how much activity they are doing) and objective actigraphy outputs (direct measure of activity).

At the beginning and end of the study participants will wear a multiaxial activity monitor (McRoberts MoveMonitor). This is worn continually at the waist for a week except while washing or bathing. The MoveMonitor records duration and intensity of any physical activity, with data uploaded securely to cloud storage. At the end of this week a one-week recall questionnaire about physical activity is completed. The c-PPAC produces an overall score that incorporates two domains of the patient experience of physical activity; “amount” and difficulty”. All three are scored from 0-100.

Difficulty is scored based on questionnaire responses. Amount is scored on a combination of the objectively measured physical activity values and the questionnaire responses. The c-PPAC is known to be responsive to telecoaching interventions (24) as well as to inhaled pharmacotherapy (25, 27). The “c” distinguishes the tool from the related d-PPAC which makes use of a daily questionnaire and is intended for use in clinical trials where participants have an electronic device to record symptoms.

The PROactive PROs have been extensively tested in practice and evaluated recently using data from seven randomised controlled trials in more 1,300 people to evaluate their internal consistency and construct validity by sex, age groups, COPD severity, country and language, as well as responsiveness to interventions, ability to detect change and minimum clinically important difference (MCID)(27). In this analysis scores covered almost the full range from 0 to 100, showed strong internal consistency after stratification, and correlated as *a priori* hypothesised with dyspnoea, health-related quality of life and exercise capacity. Difficulty scores improved after pharmacological treatment and pulmonary rehabilitation, while amount scores improved after behavioural physical activity interventions. All scores were responsive to changes in self-reported physical activity experience (both worsening and improvement) and to the occurrence of COPD exacerbations during follow-up. Using both anchor and distribution based scores an MCID of 6 for the two individual domain outcomes (amount and difficulty) and 4 for the total score could be defined(27).

The measure that we are using is the result of a five-year pan-European EU/IMI joint undertaking development programme sufficiently rigorous (by design) for it to now have

been accepted as a valid patient reported outcome measure by both FDA and EMA. This adoption by regulatory agencies provides clear evidence for the measure's robustness and clinical importance.

Objective measures of amount of physical activity are based on averaging values for days with an adequate duration of monitor use. Based on expert consensus(28) this is taken to be at least 8 hours wear between 7am and 10pm.

Spirometry The diagnosis of COPD will depend on clinical diagnosis confirmed by spirometry caried out according to ATS/ERS guidelines. Outcomes will be FEV1

Breathlessness will be assessed using extended version of the Medical Research Council Dyspnoea Scale (eMRC)(29). Participants be asked to select which of the following descriptions they feel best applies to themselves recently. The extended version is scored 1 to 5, with 5 indicating "Too breathless to leave the house". In the extended version 5 has two subdivisions; 5a independent in washing and/or dressing and 5b dependent in washing and dressing.

Health status - The COPD assessment (CAT) score will be administered to evaluate quality of life – this 8 item symptom score has been shown to be responsive both to exacerbations and to pulmonary rehabilitation.(30, 31) Each item is scored 0-5 giving a score from 0 to 40 with 40 being the worst possible health status. The EQ-5D-5L will be used to assess generic health-related quality of life and the FACIT score as a measure of fatigue.

ECG: a twelve lead ECG will be performed after 5 minutes rest on a couch. Rhythm, heart rate, axis and QTc interval will be recorded.

Body mass index - This is calculated as weight in kilos divided by height in meters squared. Height measured at the start of the study will be used for both measures. Electronic scales will be used to measure weight.

Exercise capacity will be assessed using an endurance shuttle walk test (ESWT) (32). An initial incremental shuttle walk test (ISWT) will be performed to assess exercise capacity(33). This assesses how far and fast an individual can walk without stopping for a rest, by using a series of time signals. Participants wear a pulse oximeter to measure oxygen saturations and heart rate during the exercise. They sit still for 5 minutes before the tests starts to assess resting levels. During the test they will be asked to walk around two cones spaced 9 metres apart. They begin by walking at a very slow pace; set by a beep. The goal is to walk the 10-metre course aiming to turn around a cone at the first beep, and around the second cone at the next beep. The beeps gradually get faster, which increases the pace needed to keep up. The test has 12 levels each lasting 1 minute with a minimum walking speed of 1.2 miles per hour, up to a maximum of 5.3 miles per hour. The test is stopped when they are unable to keep up with the beeps because of breathlessness or fatigue. Participants should wear comfortable, loose clothing and sensible shoes for the test and not exercise energetically for at least 2 hours before the test and also try to avoid eating a large meal before it. Subjects will perform a test walk and then a second walk and the best value taken as there is a learning effect.

The endurance shuttle walk test (32) is an accepted standard part of clinical evaluation in patients with lung disease. It measures the time at which a person can continue to walk between two cones at a pace set to be equivalent to 85% of their peak walking speed

established during the previous incremental tests. It is a validated endpoint in COPD and has been used previously to evaluate studies of therapeutic interventions (33, 34).

Participants first perform an initial incremental shuttle walk test. This involves walking between two cones 10m apart before a beep sounds. The gap between beeps shortens meaning that the person has to walk more quickly. The test stops when the person is unable to keep up with the pace. A practice walk is done first and the better of the two results taken. This pace is then used to establish the speed for the ESWT. The outcome is the time that the person can walk at this pace for, in seconds.

Prior to the walk the participant will rest quietly for 5 minutes after which their blood pressure will be measured. The person will receive standardised encouragement and can stop at any time if they wish. The reason for stopping will be documented

Oxygen saturation and heart rate during the ESWTs at the beginning and end of the study and the area under the oxygen saturation curve and the heart rate curve to isotime will be compared in the two study conditions.

All walking tests will be performed with the person using their prescribed ambulatory oxygen flow rate.

Endothelial function This will be measured using the EndoPAT-RHI system (Itamar Medical) which is an FDA approved device used for the non-invasive assessment of endothelial dysfunction. It measures pulse wave amplitude (PWA) using finger plethysmography peripheral arterial tonometry (PAT). Measurements will be carried out in the non-dominant arm after a 10-min rest in the supine position in a quiet, temperature-controlled room (21–24°C). Changes in arterial tone are elicited by creating a down-stream hyperemic response induced following a standard 5-minute occlusion of the brachial artery using a blood pressure cuff. The occlusion pressure was at least 60 mmHg above the systolic blood pressure (minimally 200 mmHg, and maximally 300 mmHg). Measurements from the contra-lateral arm are used to control for concurrent non-endothelial dependent changes in vascular tone to derive an index of endothelial function.

Each recorded test consisted of 5 minutes of baseline measurement, 5 minutes of occlusion measurement, and 5 minutes post-occlusion measurement (hyperaemic period). Outcome measures are the Reactive hyperaemia index (RHI) and PAT-Augmentation Index (AI@75)

Reactive hyperaemia index (RHI) measures of endothelial dilation function. A computerized automated algorithm has been generated to automatically calculate the RHI value by dividing post-PWA by the pre-occlusion value of PWA of the same arm, normalized to the control arm, and then multiplied by baseline correction factor (35).

The PAT-Augmentation Index (AI@75). Beat-by-beat PWA which is filtered, amplified, and graphically displayed on a computer screen. A computerized algorithm has automatically identified peak pressures and inflection point(36). The PAT-AI is then calculated by averaging the PWA data over 3.5 minutes using the following formula: PAT-AI = (augmentation pressure (ΔP)/ pulse pressure) × 100, where P1 = pulse pressure and P2 = pressure corresponding to the inflection point. These values are then corrected for a standard heart rate of 75 beats/min (AIx75).

5.7 Laboratory Evaluations

Blood samples will be obtained from participants for Full Blood Count (FBC, U&Es, BNP, ADMA, Endothelin 1 and Prostacyclin, NOx at baseline and at visit 3.

(i) Haematology

Full blood count will be performed by the local clinical laboratory using standard methods

(ii) Biochemistry

Urea and electrolytes (and calculated creatinine clearance) as well as BNP will be performed by the local clinical laboratory using standard methods

(iii)Exploratory / Research samples

Blood samples will also be taken to measure markers of vascular function including, but not limited to ADMA, L-arginine, endothelin-1, prostacyclin and NOx. In the case of NOx, levels will be measured primarily as an indicator of the effects of nitrate intake from the BRJ on circulating levels in the body. Samples will be processed at local labs and then analyses will be performed at Professor Jane Mitchell's lab at Imperial College.

Endothelial dependent vasodilation is regulated in part by the release of three endothelial derived hormones. These are (i) endothelial derived NO, (ii) endothelin-1 and (iii) prostacyclin. NO and prostacyclin are vasodilator hormones while endothelin-1 is a vasoconstrictor.

Endothelial derived NO is formed by the enzyme NOSIII from the substrate, L-arginine. The body produces a natural inhibitor of NOS, a compound called Asymmetric dimethylarginine (ADMA).

ADMA is an analogue of L-arginine and acts as a competitive inhibitor at the active site of NOS. A low Arginine/ADMA ratio is associated with the presence of vascular disease or endothelial dysfunction(37). Levels of ADMA and arginine can be measured in plasma or serum by ELISA.

Endothelin-1 is a peptide which can be measured in plasma or serum where increased levels have, in some studies, been associated with cardiovascular disease, pulmonary hypertension(38). Levels of endothelin-1 can be measured in plasma or serum by ELISA.

Prostacyclin is a cardioprotective lipid mediator with potent anti-platelet effects and which acts as a vasodilator in some blood vessels. Prostacyclin is very short lived, but its levels can be assessed by measuring its breakdown product 6-ketoPGF1alpha by ELISA. The association between plasma/serum levels of prostacyclin and human disease is less clear than for ADMA and endothelin-1.

Nitrate/nitrite (NOx)
NO is released endothelial and other cells is rapidly oxidized to nitrite and nitrate in blood. The total level of nitrite and nitrate in a plasma/serum sample is often referred to as NOx and can be measured using a colorimetric assay kit. In plasma/serum samples levels of nitrate/nitrite are in the low to mid micromolar range but these are increased in samples from people taking nitrate supplements.

To measure biomarkers, 10 mL venous blood taken into EDTA and lithium heparin tubes by venepuncture through a butterfly needle. To obtain serum and plasma, a member of the local research team will collect the samples and centrifuged at 3500 rpm for 10 minutes. Then, samples will be stored at -80C till the analyses.

Serum arginine and ADMA levels will be measured by DLD ELISA with a programmable fluorescence detector (39). These analyses will be carried out by Professor Jane Mitchell's team at Imperial College London(40).

(iv) Sample storage and analysis

Baseline and visit 3 (FBC, U&E/BNP) will be transferred to local laboratories immediately for analysis. Blood samples for Nitrate/nitrite, endothelin 1 and prostacyclin will be processed at the local laboratories, stored and then transferred to Professor Jane Mitchell's lab at Imperial College London for analysis.

Further details will be defined in study specific guidelines for sample collection and handling.

Samples will be kept beyond the end of the trial and stored in accordance with the Human Tissue Act. These will be banked in a HTA licensed Imperial College Healthcare Tissue Bank for use in future ethically approved research, except for samples whose' participants did not consent to this on their consent form – these will be safely disposed of.

(v) Incidental findings

Incidental findings will be reviewed by the research team referred to the patient's clinical team and General Practitioner as appropriate.

6. INTERVENTION



The intervention is a commercially available concentrated NO_3^{--} rich BRJ (NR-BRJ) (98%) drink cut with organic lemon juice (2%), containing 0.4 g, 6.4 mmol of NO_3^- (70mL Beet-It® SPORT shot, James White Drinks, Ipswich, UK).

The placebo beetroot juice is also produced by James White Ltd, who have a long history of manufacturing this placebo beverage for research purposes. It is 70mL of the same beverage in identical packaging from which the nitrate has been removed by a standardised method of passing the juice, prior to pasteurisation, through an ion exchange column, containing Purolite A520E which specifically exchanges NO_3^- with chloride(6, 41) This creates an otherwise identical nitrate free beverage The placebo-BRJ (PL-BRJ) is identical in appearance, taste and smell, and also causes beeturia (orange to red discolouration of urine). This allows for the study to be double-blind.

We and others have shown that the placebo juice causes no increase in blood nitrate or nitrite levels and no increase in exhaled NO levels(6, 9). The normal juice does increase these parameters, substantially.

We have independently tested both products confirming the nitrate content of the “active” juice and the absence of nitrate in the placebo. We found that the NO_3^- content, mean (SD) of 140mls of the active beetroot juice was 120.4 (5.3) mM and the NO_3^- content of the placebo was close to zero; 55.05(0.68) µM.

The specific beetroot juice intervention from James White Ltd has been chosen because it is an established, commercially available product that is a source of dietary nitrate and because they produce a matched placebo for research purposes which allows us to ensure double-blinding.Daily rather than intermittent dosing (as used in ON-EPIC) was chosen to aid compliance.

Participants will consume 70mls of one or other drink each morning, according to study allocation.

7. WITHDRAWAL FROM THE STUDY AND ADVERSE EVENTS

7.1 Permanent Discontinuation of Study Intervention and Withdrawal from Study

(i) Permanent discontinuation of study intervention

Participants may discontinue study intervention for the following reasons:

- At the request of the participant.
- Adverse event/ Serious Adverse Event
- If the investigator considers that a participant's health will be compromised due to adverse events or concomitant illness that develop after entering the study.

(ii) Withdrawal from Study

Withdrawal from the study refers to discontinuation of study intervention and study procedures and can occur for the following reasons:

- Participant decision
- Loss to follow-up

(iii)Procedures for Withdrawal from Study

If a participant permanently discontinues the trial intervention, they will be invited to continue to attend trial visits if possible, to allow for collection of key outcome and safety data.

If a participant withdraws from trial procedures, an assessment must be made as to whether trial data and samples collected to date can be retained and analysed for the trial.

The decision to withdraw from further trial procedures will be documented on the electronic case report form (eCRF) and in the medical notes.

If the participant withdraws consent to further be contacted at all for the study purposes, this will be documented on the electronic case report form (eCRF) and in the medical notes. Data up to the time of withdrawal can be included in the study if anonymised.

7.2 Adverse Events (AE)

An AE is any untoward medical occurrence in a patient or clinical trial participant. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, whether or not considered related to the trial protocol.

7.3 Adverse Event recording

For the purposes of the study, AEs will be followed up according to local practice until the event has stabilised or resolved, or the Follow-up Visit, whichever is the sooner. SAEs will be recorded throughout the study.

(i) Severity of Adverse Events

Mild: Awareness of event but easily tolerated
Moderate: Discomfort enough to cause some interference with usual activity
Severe: Inability to carry out usual activity

(ii) Causality of Adverse Events

Unrelated: No evidence of any causal relationship
Unlikely: There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).
Possible: There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).
Probable: There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definite: There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

7.4 Abnormal Laboratory Test Results

All clinically important abnormal laboratory test results occurring during the study will be recorded as adverse events. The clinically important abnormal laboratory tests will be repeated at appropriate intervals until they return either to baseline or to a level deemed acceptable by the investigator and the clinical monitor, or until a diagnosis that explains them is made.

7.5 Serious Adverse Events (SAE)

An SAE is defined as any event that

- Results in death;
- Is life-threatening*;
- Requires hospitalisation or prolongation of existing inpatient's hospitalisation**;
- Results in persistent or significant disability or incapacity;
- Is a congenital abnormality or birth defect;

* "Life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

** “Hospitalisation” means any unexpected admission to a hospital department. It does not usually apply to scheduled admissions that were planned before study inclusion or visits to casualty (without admission).

Medical judgement should be exercised in deciding whether an adverse event/reaction is serious in other situations. Important adverse events/reactions that are not immediately life-threatening, or do not result in death or hospitalisation but may jeopardise a participant or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

7.6 Reporting of SAEs

Reporting of all SAEs, with the exception of acute exacerbations of COPD, occurring during the study must be performed within 24 hours of the investigator becoming aware of them.

Active monitoring of participants after the end of the trial is not required, but if the investigator becomes aware of safety information that appears to be related to the trial, involving a participant who participated in the study, even after an individual participant has completed the study, this should be reported to the Sponsor within 15 days of the investigator becoming aware of the event.

All SAEs will be reviewed by the Chief Investigator or a designated medically qualified representative to confirm expectedness and causality.

Reporting of SAEs and review by the CI will be via the trial data collection system (eCRF).

(i) Related SAEs

Related: resulted from administration of any of the research procedures

(ii) Unexpected SAEs

Unexpected: type of event is not listed in the protocol as an expected occurrence.

(iii) Reporting of SAEs that are related and unexpected

SAEs that are *related and unexpected* should be notified to the relevant REC and to the Sponsor within 15 days of the investigator becoming aware of the event.

Follow up of participants who have experienced a related and unexpected SAE should continue until recovery is complete or the condition has stabilised. Reports for related and unexpected SAEs should be unblinded prior to submission if required by national requirements.

(iv) Annual reporting of Serious Adverse Events

Annual Progress reports will be submitted to the Sponsor and the Ethics Committee in accordance with local requirements. The Annual Progress Report will detail all SAEs recorded.

7.7 Reporting urgent safety measures

If any urgent safety measures are taken the CI/Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the relevant REC of the measures taken and the circumstances giving rise to those measures.

8. STATISTICAL ANALYSES

8.1 Sample Size and power considerations

Using data from the PROactive COPD consortium, in the most limited COPD population studied, the standard deviation for the PPAC score was 8 with a median value of 54 (27). To have a 90% chance of identifying a 6 point difference (the MCID) in the difficulty domain, at a two-sided 5% level of significance, requires a sample size of 38 in each group, 76 in total. To allow for 25% dropout we will enrol 102 participants.

8.2 Statistical Analysis

Data will be entered into a validated eCRF developed by ICTU. Analysis will be carried out by a trial statistician at the Imperial College Clinical Trials Unit. Continuous variables will be presented as means and standard deviations if normally distributed, and as medians and inter-quartile ranges for skewed data, whilst categorical variables will be presented as frequencies and percentages. Normality will be checked, and appropriate transformation performed if not normally distributed. All statistical tests will be two-tailed with a 5% significance level.

A detailed description of all the analyses will be given in a detailed statistical analysis plan (SAP) will be prepared and finalised prior to database lock. Any deviations from the SAP will be justified and documented in the final report.

(i) Analysis populations

The primary analysis will be performed according to the intention to treat principle, including all participants who are randomised to a study arm, according to their allocated arm, regardless of treatment received.

A Per-Protocol (PP) population will also be defined removing participants who are non-compliant with their treatment. Non-compliance will be defined as consuming <70% of treatment doses.

(ii) Primary Endpoint Analysis

The primary endpoint difficulty domain of the c-PPAC score. This will be analysed using quantile regression with baseline c-PPAC as a covariate, treatment (Beetroot juice, Placebo) and the stratification factors sites, oxygen use (LTOT vs Ambulatory oxygen only) and eMRC dyspnoea score (≤4 vs >4) as fixed effects.

(iii)Secondary Endpoints Analysis

The same methodology will be used for the analysis of secondary and mechanistic endpoints using quantile regression for discrete or non-normal continuous outcomes, ANCOVA for normally distributed continuous outcomes, and logistic regression for categorical outcomes.

Secondary endpoints:

Physical activity

Amount domain of c-PPAC score

Total c-PPAC score

Step count

Health status

CAT score

FACIT SCORE

EQ-5D-5L

Exercise capacity

ESWT time

ESWT AUC for O_2 saturation

Vascular parameters

SBP/DBP/MAP

Endopat RHI

Endopat AI

Sleep disturbance

Sleep movements

Times out of bed

(iv)Mechanistic endpoints

Arg/ADMA ratio
Endothelin-1
Prostacyclin
BNP
NOx

REGULATORY, ETHICAL AND LEGAL ISSUES

8.3 Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformity with the *7th* revision of the 1964 Declaration of Helsinki.

8.4 Good Clinical Practice

The study will be conducted in accordance with the guidelines laid down by the International Conference on Harmonisation for Good Clinical Practice (ICH GCP E6 guidelines).

8.5 Research Ethics Committee (REC) Approval

(i) Initial Approval

Prior to the enrolment of participants, the REC must provide written approval of the conduct of the study at named sites, the protocol and any amendments, the Participant Information Sheet and Consent Form, any other written information that will be provided to the participants, any advertisements that will be used and details of any participant compensation.

(ii) Approval of Amendments

Proposed amendments to the protocol and aforementioned documents must be submitted to the REC for approval as instructed by the Sponsor. Amendments requiring REC approval may be implemented only after a copy of the REC's approval letter has been obtained.

Amendments that are intended to eliminate an apparent immediate hazard to participants may be implemented prior to receiving Sponsor or REC approval. However, in this case, approval must be obtained as soon as possible after implementation.

Amendments will be developed and approved by the Trial Steering Committee.

(iii) Annual Progress Reports

Annual Progress Reports will be submitted to the Research Ethics Committee (REC) and the Sponsor in accordance with local / national requirements. The Annual Progress Report will also detail all SAEs recorded.

(iv) End of Trial Notification

The REC will be informed about the end of the trial, within the required timelines.
The end of trial notification will be submitted within 90 days of the end of trial definition being met. In the event of a premature halt of the trial, the timeframe is 15 days, and the reasons should be clearly explained in the notification.

8.6 HRA approval

Health Research Authority (HRA) approval will be obtained prior to starting the study. Each participating site will confirm capacity and capability prior to commencing.

The HRA and all participating sites also need to be notified of all protocol amendments to assess whether the amendment affects the institutional approval for each site.

8.7 Non-Compliance and Serious Breaches

All protocol deviations and protocol violations will be reported via the eCRF/CRF and reviewed by the Chief Investigator and reported to the ICTU QA manager on a monthly basis. Protocol violations will be reported to the Sponsor.

An assessment of whether the protocol deviation/violation constitutes a serious breach will be made.

A serious breach is defined as:

A breach of the conditions and principles of GCP in connection with a trial or the trial protocol, which is likely to affect to a significant degree:

- The safety or physical or mental integrity of the UK trial participants; or
- The overall scientific value of the trial

The Sponsor will be notified within 24 hours of identifying a likely Serious Breach. If a decision is made that the incident constitutes a Serious Breach, this will be reported to the REC within 7 days of becoming aware of the serious breach.

8.8 Insurance and Indemnity and Sponsor

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

Imperial College London will act as the main Sponsor for this trial. Delegated responsibilities will be assigned to the NHS trusts taking part in the trial.

8.9 Trial Registration

The study will be registered on a trial database in accordance with requirements of the International Committee of Medical Journal Editors (ICMJE) regulations.

8.10 Informed Consent

Informed consent will be obtained from all participants using REC approved Participant Information Sheet (s) (PIS) and Consent Forms (CF) during the baseline visit.

The participant will be informed of about the trial by responsible clinician or a member of the research team and given a copy of the PIS. Informed subjects will be given an adequate amount of time to consider their participation in the trial. If the subject decides to participate in the trial they will be asked to sign the CF which will then be countersigned by the responsible clinician/researcher/ The patient will obtain one copy of the signed CF. Another copy will be place in the participant's medical records whilst the original will be retained in the participant's research record, at site.

The right of the participant to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time from the protocol intervention without giving reasons and without prejudicing further treatment.

8.11 Contact with General Practitioner

The investigator will inform the participant's General Practitioner by letter that the participant is taking part in the study provided the participant agrees to this, and information to this effect is included in the Participant Information Sheet and Informed Consent. A copy of the letter should be filed in the Investigator Site File.

8.12 Participant Confidentiality

The investigator must ensure that the participant's confidentiality is maintained. On the CRF or other documents submitted to the Sponsors, participants will be identified by a participant ID number only. Documents that are not submitted to the Sponsor (e.g., signed informed consent form) should be kept in a strictly confidential file by the investigator.

The investigator shall permit direct access to participants' records and source document for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, NHS, Regulatory Authorities and RECs.

8.13 Data Protection and Participant Confidentiality

The investigators and study site staff will comply with the requirements of the Data Protection Act 2018 concerning the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

8.14 End of Trial

The end of the trial will be defined as entry of the final data item following last patient last visit.

8.15 Study Documentation and Data Storage

The investigator must retain essential documents until notified by the Sponsor, and for at least ten years after study completion. Participant files and other source data (including copies of protocols, CRFs, original reports of test results, correspondence, records of informed consent, and other documents pertaining to the conduct of the study) must be retained. Documents should be stored in such a way that they can be accessed/data retrieved at a later date. Consideration should be given to security and environmental risks.

No study document will be destroyed without prior written agreement between the Sponsor and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

9. DATA MANAGEMENT

9.1 Source Data

Data will be collected into a worksheet including ECGs, device printouts and treated as source data. This will then be transcribed into eCRF.

9.2 Language

eCRFs will be in English. Generic names for concomitant medications should be recorded in the eCRF wherever possible. All written material to be used by participants will use vocabulary that is clearly understood and be in the language appropriate for the study site.

9.3 Database

Trial data will be collected on an electronic case report form (eCRF) - OpenClinica. The principal means of data collection from participant visits will be Electronic Data Capture

(EDC) via the internet using the OpenCllinica database. Data is entered into the EDC system by trained site personnel. All data recorded in the eCRF will be signed off by the Investigator or his/her appropriate designee. All changes made following initial submission of data will have an electronic audit trail with a date. Specific instructions and further details will be outlined in the study specific eCRF manual.

9.4 Randomisation and unblinding

Randomisation and unblinding will be carried out using web-based randomisation and Electronic Data Capture (EDC) system, called OpenClinica eCRF. Randomisation will be stratified by site, oxygen use (groups) and eMRC dyspnoea score (group and will be blocked to ensure equal allocation to each arm.

9.5 Archiving

All trial documentation, including that held at participating sites and the trial coordinating centre, will be archived for a minimum of 10 years following the end of the study.

10. STUDY MANAGEMENT STRUCTURE

The trial will be managed by the United Kingdom Clinical Research Collaboration (UKCRC) registered Imperial Clinical Trials Unit (ICTU).

10.1 Trial Steering Committee

A Trial Steering Committee (TSC) will be convened including as a minimum an independent Chair, a lay person, an independent clinician, the Chief Investigator and Trial Manager. The role of the TSC is to provide overall supervision of trial conduct and progress. Details of membership, responsibilities and frequency of meetings will be defined in a separate Charter

10.2 Trial Management Group

A Trial Management Group (TMG) will be convened including the Chief Investigator, co-investigators and key collaborators, trial statistician and trial manager. The TMG will be responsible for day-to-day conduct of the trial and operational issues. Details of membership, responsibilities and frequency of meetings will be defined in separate terms of Reference.

10.3 Data Monitoring Committee

A fully independent Data Monitoring and Ethics Committee (DMEC) will be set up to monitor progress, participant safety and any ethical issues involved in this trial. They will review trial progress, recruitment rates and safety data. A separate DMEC Charter will be drawn up defining their responsibilities, frequency of meetings and reporting to the TSC.

The statistician will analyse interim data for DMEC meetings and act as data manager, in raising and resolving data queries with participating sites, via the Trial Manager. Closed DMEC reports will include recruitment, randomisation balance and stratification effectiveness, baseline characteristics, unblinding, withdrawals, compliance, concomitant medications, efficacy, mediators, and adverse events. Open DMEC and TSC reports will be provided without outcome or arm information.

10.4 Early Discontinuation of the Study

No criteria to discontinue the study early are anticipated. In case of early discontinuation of the study, the Follow-up Visit assessments should be performed for each participant, if possible.

10.5 Risk Assessment

A study-specific risk assessment will be performed prior to the start of the study to assign a risk category of 'low', 'medium' or 'high' to the trial. Risk assessment will be carried out by the ICTU QA Manager in collaboration with the Study Manager and the result will be used to guide the monitoring plan.
The risk assessment will consider all aspects of the study and will be updated as required during the course of the study.

10.6 Monitoring

The study will be monitored periodically by trial monitors to assess the progress of the study, verify adherence to the protocol, ICH GCP E6 guidelines and other national/international requirements and to review the completeness, accuracy, and consistency of the data.

Monitoring procedures and requirements will be documented in a Monitoring Plan, in accordance with the risk assessment.

10.7 Quality Control and Quality Assurance

Quality Control will be performed according to ICTU internal procedures. The study may be audited by a Quality Assurance representative of the Sponsor and/or ICTU. All necessary data and documents will be made available for inspection.

The study may be subject to inspection and audit by regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd Edition).

10.8 Peer review

The trial has undergone independent peer review via the NIHR-EME funding programme. The trial has also been reviewed by senior members of ICTU.

10.9 Patient and Public Involvement

- A public partner lead will be recruited to sit on the trial management group and to sit on the trial steering committee. A patient advisory group of six patients will be established. This group will ensure continuity, involving the same public partners through the duration of the trial. They will be supported by the PPI lead and will be offered a series of 5 PPI training sessions.
- The patient advisory group will influence the design of the trial so that it is as manageable for as diverse a group as possible so that views and suggestions represent as far as possible those of the people for whom the research is relevant. The group will guide on the way the trial is presented to potential participants in patient information documents so that these are understandable and appealing as well as advising on how trials can be advertised via social media/other networks to reach more diverse audiences.

- Involving this diverse group in dissemination will ensure the results are presented clearly and in ways appropriate for diverse groups and that these are distributed to reach as diverse an audience as possible.
- Methods of ensuring diversity and equality when recruiting will be discussed with our patient advisory group. These will include thinking about where different groups tend to access information in order to tailor communication strategies; trying to establish links and contacts with local communities; the use of different communications media, e.g. posters displayed in key places (such as community centres or religious places) and tapping into free community resources, e.g. community radio stations.

10.10 Publication and Dissemination policy

Information concerning the study, patent applications, processes, scientific data or other pertinent information is confidential and remains the property of the Sponsor. The investigator may use this information for the purposes of the study only.

It is understood by the investigator that the Sponsor will use information developed in this clinical study and, therefore, may disclose it as required to other clinical investigators. In order to allow the use of the information derived from this clinical study, the investigator understands that he/she has an obligation to provide complete test results and all data developed during this study to the Sponsor.

Verbal or written discussion of results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor.

Therefore, all information obtained because of the study will be regarded as CONFIDENTIAL, at least until appropriate analysis and review by the investigator(s) are completed.

A Clinical Study Report summarising the study results will be prepared and submitted to the REC within a year of the end of study.

The results of the study will be published in high impact peer-reviewed open access journals. This will be augmented by other dissemination strategies including blogs/editorials and press release supported by the media teams at Royal Brompton and NHLI, Imperial College London. We will ensure that the results are communicated to key relevant groups (e.g. BTS Specialist Advisory Groups on COPD and on Oxygen).

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12. REVISION HISTORY

Version	Date	Summary of changes
2.2	07-11-2024	Addition of funder logo and acknowledgement. Corrected NIHR reference number.
2.1	13-06-2024	Non-substantial amendment to add third site.
2.0	30-01-2024	Non-substantial amendment to add second site
1.2	24-02-2023	Substantial amendment to add recruitment pathways and correction to statistical analysis
1.1	11-10-2022	Non-substantial amendment
1.0	24-05-2022	Incorporated changes following sponsor review
1.0	08-04-2022	Incorporated changes requested by ICTU QA team following Protocol QC review
0.2	04-04-2022	Version amended following reviews by Protocol review group
0.1	11-02-2022	First protocol draft created

SIGNATURE PAGE 1 (CHIEF INVESTIGATOR)

The signature below constitutes approval of this protocol by the signatory, on behalf of the Protocol Development Group, and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: Dietary nitrate supplementation to enhance daily physical activity in hypoxic COPD: a randomised controlled trial

Protocol Number: 22IC7759.

Signed: _____

Professor Nicholas Hopkinson
Professor of Respiratory Medicine, Imperial College, London

Date: _____

SIGNATURE PAGE 2 (SPONSOR)

Study Title: Dietary nitrate supplementation to enhance daily physical activity in hypoxic COPD: a randomised controlled trial

The signatures below constitute approval of this protocol by the signatory.

Protocol Number: 22IC7759.

Signed: _____

Keith Boland
Research Governance and Integrity Team (RGIT)
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Date: _____

SIGNATURE PAGE 3 (STATISTICIAN)

Study Title: Dietary nitrate supplementation to enhance daily physical activity in hypoxic COPD: a randomised controlled trial

The signatures below constitute approval of this protocol by the signatory.

Protocol Number: 22IC7759.

Signed: _____

Ms Emanuela Falaschetti
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Date: _____

SIGNATURE PAGE 4 (PRINCIPAL INVESTIGATOR)

The signature of the below constitutes agreement of this protocol by the signatory and provides the necessary assurance that this study will be conducted at his/her investigational site according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: Dietary nitrate supplementation to enhance daily physical activity in hypoxic COPD: a randomised controlled trial

Protocol Number: 22IC7759.

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Signed: _____

Print Name and Title: Professor Nicholas Hopkinson

Date: _____