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RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL INVESTIGATING THE EFFECTS OF INORGANIC NITRATE IN HYPERTENSION-INDUCED TARGET ORGAN DAMAGE: PROTOCOL OF THE NITRATE-TOD STUDY

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ABSTRACT

Introduction

Arterial stiffness and left ventricular hypertrophy (LVH) are key markers of hypertensive target organ damage (TOD) associated with increased cardiovascular morbidity and mortality. We have previously shown that dietary inorganic nitrate supplementation lowers blood pressure (BP) in hypertension, however, whether this approach might also improve markers of hypertensive TOD is unknown. In this study, we will investigate whether daily dietary inorganic nitrate administration reduces left ventricular (LV) mass and improves measures of arterial stiffness.

Methods and Design

NITRATE-TOD is a double-blind, randomised, single-centre, placebo-controlled phase II trial aiming to enrol 160 patients with suboptimal BP control on 1 or more anti-hypertensives. Patients will be randomised to receive 4-month once daily dose of either nitrate-rich beetroot juice or nitrate-deplete beetroot juice (placebo). The primary outcomes are reduction in LV mass and reduction in pulse wave velocity (PWV) and central blood pressure (CBP).

The study has a power of 95% for detecting a 9g LV mass change by cardiovascular magnetic resonance (CMR) imaging (~6% change for a mildly hypertrophied heart of 150g). For PWV we have a power of > 95% for detecting a 0.6 m/s absolute change. For central systolic BP, we have a > 90% power to detect a 5.8 mm Hg difference in central systolic BP.

Secondary end-points include change in ultrasound flow mediated dilatation (FMD), change in plasma nitrate and nitrite concentration and change in BP.

Ethics and Dissemination

The study was approved by the London – City and East Research Ethics Committee (10/H0703/98). Trial results will be published according to the CONSORT statement and will be presented at conferences and reported in peer-reviewed journals.

ClinicalTrials.gov identifier: NCT03088514

Strengths and limitations of this study

- This is a randomised, placebo-controlled double-blind clinical trial assessing the potential of dietary nitrate in reducing both cardiac and vascular damage due to persistently elevated blood pressure.
- Dietary nitrate is a simple, easy to use and safe intervention that may be more acceptable to patients on long term medication for their blood pressure.
- This is a single-centre phase II study; therefore, the applicability to other centres is uncertain.

INTRODUCTION

Hypertension is the leading risk factor for cardiovascular diseases (CVD) worldwide.[1,2] In 2016, an estimated 17.9 million people died from CVDs, which represents 31% of all global deaths.[3] Approximately half of these deaths were caused by complications from hypertension.[4] Hence, novel and cost-effective therapeutic strategies continue to be sought. In this regard there has been a major emphasis to increase vegetable intake since increased consumption of a vegetable-rich diet particularly green leafy vegetables confers protection against CVD, including the lowering of BP[5,6]. One particular constituent of such vegetables that has been proposed to underlie the beneficial effects of this food group is inorganic nitrate; through bioconversion to nitrite, and then nitrite to nitric oxide (NO)[7] in the body. Importantly, provision of inorganic nitrate to hypertensive patients causes a rise in plasma nitrate and is associated with a decrease in BP.[8,9]

Dietary inorganic nitrate once ingested rapidly enters the circulation[8–10] with a proportion of this nitrate being extracted and then secreted and concentrated in the saliva.[11] Bacteria residing in the oral cavity convert this nitrate to nitrite which is then swallowed;[12] appearing soon after within the circulation. We have recently shown that daily supplementation with dietary nitrate (providing approximately 6.4 mmol nitrate daily) for 4 weeks was associated with robust, sustained and clinically meaningful reductions in BP (measured by clinic, ambulatory and home methods) of ~8/4 mm Hg.[13] However, persistent hypertension leads to both vascular and cardiac remodelling and whether inorganic nitrate-induced reductions in BP might be associated with improvements in either of these in the long term is unknown. Experimentally in patients one can assess this potential through the measurement of arterial stiffness and LV mass respectively, with increases of either conferring increased CV risk. Arterial stiffness (and its inverse measure, distensibility) refers to the decreased elasticity that develops in arteries as a consequence of vascular remodelling resulting from alterations of the fibrous components of the extracellular matrix (including elastin and collagen).[14] Importantly, increased arterial stiffness, as measured using carotid-femoral PWV,[15] is a strong predictor of CV events,[16] and there have been suggestions that drug development targeting this phenomenon is likely to provide a new genre of therapeutics in the combat against CVD.[17] Additionally, an increased central pulse pressure occurs when the large conduit blood vessels lose their elasticity and become less able to accommodate all of the blood ejected from the heart. This increase in pressure results in an increase in central systolic BP and causes an increase in the stress imposed on the left ventricle, which in turn can result in left ventricular hypertrophy (LVH).[18] Elevated LV mass is an initial compensatory mechanism to normalise wall stress due to elevated BP.

However, this cardiac remodelling eventually becomes maladaptive with an increase in myocardial oxygen consumption, cardiac chamber dilation, reduced LV contractility and progressive deterioration to heart failure.[19] The measure of LVH as a prognostic indicator of adverse outcome was first convincingly identified in the Framingham study,[20] and numerous studies since have confirmed the strong relationship between LVH and CV events and mortality.[21,22] Perhaps, more importantly, LV mass reduction is associated with further improvements in CV outcomes.[23,24]

It has been proposed that insufficient supply of endogenous NO likely contributes to the progression and worsening of arterial stiffness and LVH.[25] Pre-clinical studies have demonstrated that an elevation of systemic nitrite levels reduces cardiac hypertrophy in mice.[26] Moreover, administration of inorganic nitrate reduces PWV healthy volunteers and hypertensive patients in short term studies.[13,27]

The objective of this study is to determine whether a dietary nitrate intervention might elevate the levels of NO in the body sufficiently to alter both heart and blood vessel remodelling in hypertensive patients with suboptimal BP control.

METHODOLOGY

Trial objectives

Aims of research: We wish to test the hypothesis that dietary supplementation of inorganic nitrate (in beetroot juice) elevates circulating nitrate and nitrite levels ultimately delivering NO to the vasculature and thereby improving endothelial, vascular and cardiac function in patients with treated yet uncontrolled hypertension. Specifically, the aim is to investigate whether prolonged dietary nitrate ingestion in hypertensive patients with suboptimal BP control, can cause reductions in LV mass and PWV.

Participant selection

This is a single-centre study, in which patients will be identified and recruited at Barts Health NHS Trust. In addition, other participant identification centres will be used to identify suitable participants via the NIHR Clinical Research Network and local NHS Trusts.

Original hypothesis

Dietary inorganic nitrate ingestion, in addition to existing pharmacological therapy, reduces BP leading to reductions in LV mass and arterial stiffness.

Primary end points

The primary end points are change in LV mass, determined using cardiovascular magnetic resonance imaging (CMR) (NITRATE-LVH arm) and a change in central systolic BP and PWV (NITRATE-CBP arm).

Secondary end points

Secondary endpoints are change in ultrasound determined brachial artery flow-mediated dilation (FMD), change in plasma nitrate and nitrite levels and a change in peripheral BP.

Exploratory end points

Exploratory end points for cardiac imaging include a change in aortic distensibility, LV systolic and diastolic function, LV volumes and ejection fraction, left atrial volumes and function and markers of myocardial fibrosis and oedema. We will assess changes in 12-lead ECG parameters of LVH. With regards to BP, we will assess for changes in BP variability, BP control rate and BP circadian pattern. We will also perform analysis of the salivary microbiome. Finally, we will assess changes in quality of life score (EQ-5D questionnaire) and biochemical measures e.g. urine albumin excretion, cholesterol fractions, B-natriuretic peptide (BNP) and troponin T.

Inclusion criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

1. Patients will be enrolled following an informed consent.
2. Aged 18-80 years.
3. The study subjects will be hypertensive with evidence of difficulty treating to target BP (daytime ABPM 135-170 and/or 85-105 mm Hg) on 1 or more antihypertensive agent, with insufficient efficacy or intolerance of medications.
4. For the NITRATE-LVH arm, echocardiographic evidence of LVH (LV mass indexed to body surface area (BSA); males > 115 g/m^2; females > 95 g/m^2).
5. Patients will have been established on an antihypertensive treatment regime for at least 1 month by the time of participation in the study and will not require changes in pharmacological intervention for the duration of the trial.

Exclusion criteria

Unless specified, a subject will not be eligible for inclusion in this study if any of the following criteria apply:

1. History of chronic viral hepatitis (including presence of hepatitis B surface antigen or hepatitis C antibody), or other chronic hepatic disorders.
2. History of increased liver function tests (ALT, AST) due to acute or chronic liver conditions, 3x above the upper limit of normal or bilirubin 1.5x above the upper limit of normal at screening.
3. Renal impairment with creatinine clearance (eGFR) of < 50 ml/min at screening.
4. Patients with diabetes mellitus, defined by previous history of diabetes or HbA1c > 6.5% (> 48 mmol/mol) at screening.
5. Subjects with LDLc, > 7.5 mmol/l and/or triglyceride level > 10 mmol/l.
6. History of heart failure defined as NYHA class II - IV or those with known LV dysfunction (LV ejection fraction < 40%) regardless of symptomatic status.
7. History of malignancy within the past 5 years, other than non-melanoma skin cancer.
8. Current life-threatening condition other than vascular disease (e.g. very severe chronic airways disease, HIV positive, life-threatening arrhythmias) that may prevent a subject from completing the study.

9. Use of an investigational device or investigational drug within 30 days or 5 half-lives (whichever is longer) preceding the first dose of study medication.
10. Subjects who will commence or who are likely to commence regular treatment with non-steroidal anti-inflammatory drugs (NSAIDs) (other than aspirin), from screening until study completion.
11. Any non-stable dosing of ongoing medication regimens throughout the study trial.
12. Drug abuse within the past 6 months.
13. The subject has a three-month prior history of regular alcohol consumption exceeding an average weekly intake of > 28 units (or an average daily intake of greater than 3 units) for males, or an average weekly intake of > 21 units (or an average daily intake of greater than 2 units) for females. 1 unit is equivalent to a half-pint (284mL) of beer/lager; 25mL measure of spirits or 125mL of wine.
14. Any other subject whom the Investigator deems unsuitable for the study (e.g. due to other medical reasons, laboratory abnormalities, expected study medication noncompliance, or subject's unwillingness to comply with all study-related study procedures).
15. Subjects with rheumatoid arthritis, connective tissue disorders and other conditions known to be associated with chronic inflammation (e.g. Inflammatory Bowel Disease).
16. Subjects with any acute infection, or recent systemic (oral or IV) antibiotics within 1 month of screening, or significant trauma (burns, fractures).
17. Subjects who have donated more than 500 mL of blood within 56 days prior to the study medication administration.
18. Self-reported use of anti-microbial mouthwash or tongue scrapes.
19. Concomitant xanthine oxidase inhibitors (such as allopurinol).
20. Known history of significant claustrophobia, previous intolerance of CMR imaging or known (or suspected) incompatible metallic implant.
21. Pregnancy.
22. Allergy to gadolinium-based contrast agents used for CMR.
23. Patients with known LVH caused by another established pathology diagnosed prior to or at screening e.g. severe aortic stenosis, hypertrophic cardiomyopathy, amyloidosis and Fabry disease.

Exceptions to the exclusion criteria:

- For criteria 18, patients can enter the trial if they discontinue the use of anti-microbial mouthwash for the duration of the clinical trial.

- Criteria 20 and 22 do not apply to participants who will not have a CMR scan in the NITRATE-CBP arm.

Study design and intervention

This is a prospective double-blind, placebo-controlled, clinical study. A total of 160 patients (male and female, age 18–80) with hypertension as per requirements indicated above will be recruited. Figure 1 shows a summary of the study scheme. Patients will be stratified at screening into 2 arms. Participants with LVH at screening echocardiography will enter the NITRATE-LVH arm (n=80) whilst those who do not have LVH but satisfy the other eligibility criteria will enter the NITRATE-CBP arm (n=80). LVH is defined by increased LV mass determined by acquiring a parasternal long axis image and subsequently measured for the interventricular septal wall thickness, end-diastolic LV cavity diameter and posterior LV wall thickness according to published guidelines.[28] LV mass is calculated as previously described and indexed to body surface area (BSA).[29]

All visits will take place at the William Harvey Heart Centre, Queen Mary University of London and Barts Heart Centre, St Bartholomew's Hospital, Barts Health NHS Trust. Patients will be randomised to receive 70 mL of a beetroot juice concentrate containing ~6 mmol nitrate or nitrate-depleted placebo juice concentrate (James White Drinks, UK) control. The volunteers will start taking their daily dose after completion of their baseline visit (visit 1) for 16 weeks. Patients will be advised to take their dose of juice at the same time each day, preferably in the morning with their breakfast. Patients will be provided with dietary advice in relation to the calorific content of interventions (both ~100kcal).

Randomisation and blinding process

Patients will be block randomised on a 1:1 basis to receive either dietary nitrate or placebo, using a binary random number sequence (http://www.random.org). Treatment assignment for volunteers in the dietary nitrate and placebo groups will remain blinded until data lock and statistical analysis at the end of the study. If unblinding is required, the chief investigator for the study will be informed. A list of the unblinded treatments will be kept in a secure location at the Barts Cardiovascular Clinical Trials Unit (CVCTU). The unblinding procedure will be available at all times.

Patient involvement

The NIHR Barts Biomedical Research Centre Patient and Public Advisory Group reviewed this protocol prior to submission to the Research Ethics Committee (REC). In addition, 20 previous Barts Health

NHS Trust patients were surveyed regarding aspects of trials involving dietary interventions, which informed this protocol.

Methods to be used

Blood, saliva and urine analysis

In this study, venous blood will be taken using a 21-gauge butterfly needle at each visit. Blood samples will be centrifuged immediately and plasma separated. The plasma samples will be snap frozen in liquid nitrogen and stored at -80°C. These samples will be used for the purposes of making biochemical measurements and will be discarded once used. Similarly, saliva and urine will be collected at each visit. Saliva will be centrifuged, and a pellet generated. This pellet contains oral bacteria that have dislodged from the oral cavity. This pellet will be frozen for later analysis of oral bacteria by second-generation genome sequencing.

Nitrate and nitrite levels in saliva, blood and urine samples will be determined using ozone chemiluminescence as previously described.[30,31] In brief, total nitrate and nitrite concentration (termed 'NOx') will be determined by adding samples to 0.1mol/L vanadium (III) chloride in 1M hydrochloric acid refluxing at 95°C under nitrogen. Nitrite concentration will be determined by the addition of samples to 0.09 mol/L potassium iodide in glacial acetic acid under nitrogen at room temperature. Nitrate concentration will be calculated by the subtraction of [nitrite] from [NOx]. All measurements will be conducted by an individual blinded to the intervention.

Pulse wave analysis and pulse wave velocity

Pulse wave analysis (PWA) and PWV are measures of arterial stiffness, which will be determined by a non-invasive Vicorder device (Skidmore Medical Limited, Bristol, UK). For PWA, the pulse wave will be recorded from the brachial cuff applied to the non-dominant arm. For PWV, the pulse wave will be simultaneously recorded from the carotid and femoral site using an oscillometric method. A small, inflatable neck pad will be placed directly over a carotid artery and secured around the neck by a Velcro tab and a cuff will also be placed around the patient's ipsilateral upper thigh. Both carotid and femoral cuffs will be simultaneously inflated automatically to 65 mm Hg and the corresponding oscillometric signal from each cuff digitally analysed to extract the pulse time delay. To estimate the aortic length, the distance between the sternal notch and the thigh cuff will be measured. From these measurements, PWV can be derived as PWV = aortic distance/pulse time delay.[32]

Flow-mediated dilation

FMD will be used to non-invasively assess endothelial function, using vascular ultrasound to measure the increase in brachial artery diameter in response to increased flow[33] as previously described.[34] A high-resolution external vascular ultrasound Siemens/Acuson Sequoia C256 Colour Doppler with a 7.0-MHz linear-array transducer supported by a stereotactic clamp will be used to assess the vessel diameter in the right arm. The vessel will be scanned in longitudinal section and the centre will be identified when the clearest views of the anterior and posterior artery walls have been obtained. Images will be magnified with a resolution box function and images of the brachial artery acquired continuously using semi-automated edge detection software (FMD Studio, Quipu s.r.l, Pisa, Italy) and analysed in real time. An automatic mathematical contour tracking operator locates the pulsed-wave Doppler of the brachial artery which will be used to measure the diameter and blood flow velocity continuously for 1 minute at baseline, then during 5 minutes of reduced blood flow (induced by inflation to 300 mm Hg of a pneumatic cuff placed on the right forearm site distal to the segment of artery being analysed), and finally for a further 5 minutes during reactive hyperaemia following cuff release. FMD is defined as the maximum percentage increase in vessel diameter during reactive hyperaemia. This procedure will be performed at visit 1 (baseline) and visit 3 (16 weeks). In some volunteers, following FMD, 0.4 mg of sublingual GTN (glyceryl trinitrate) will be administered at visit 1 and visit 3 to determine whether changes in FMD responses following intervention might be due to changes in smooth muscle reactivity.

Transthoracic echocardiography

At visit 1 and 3, echocardiography is also performed to assess LV diastolic function. Images will be acquired for measures of LV diastolic function including left atrial end-diastolic volumes, pulmonary vein s/d ratio, mitral valve E/A ratio and E/e'.[28,35] Images will be acquired and analysed by an operator accredited by the British Society of Echocardiography.

CMR

The most accurate and reproducible measurements of LVH are made by CMR.[36] At visits 1 and 3, CMR in the NITRATE-LVH arm and those that consent in the NITRATE-CBP arm will be used to assess LV mass, volumes and ejection fraction, T1 and T2 mapping, patterns of late gadolinium enhancement (LGE), aortic distensibility and PWV. Participants will have a peripheral venous cannula inserted and gadolinium injected at doses up to 0.2 mmol/kg.

To allow a thorough assessment of cardiac function, the following imaging sequences will be undertaken:

- Scout images, including black and white blood transverse slices of the thorax
- Long axis cines (4-chamber, 2-chamber, LVOT-1, LVOT-2, aortic valve short axis)
- Short axis cine stack
- T1 and T2 mapping
- LGE in the 4-chamber, 2-chamber, 3-chamber views and short axis stack
- Post contrast T1 mapping for extra-cellular volume (ECV) quantification
- Sagittal aortic views and aortic flow sequences for assessment of aortic distensibility and PWV.

Short axis images will be acquired using standard sequences i.e. 10 – 12 slices of 8mm thickness with 2mm gap to achieve whole LV coverage. This is undertaken with the patient in held expiration. Cine and LGE short axis stacks will be acquired using identical scanning geometry to assure correct image registration.

The following offline parameters will be measured:

- Presence of significant extra cardiac pathology
- Left ventricular ejection fraction, volumes and mass
- Distribution of LGE
- Analysis of pre and post contrast T1 mapping and T2 mapping. Short axis images will be segmented according to the AHA model as previously described[37]
- Aortic distensibility and pulse wave velocity.

For the analysis of study outcomes, each study will be anonymised by a third party. This will be separate from the study ID to avoid bias between pre- and post-treatment studies. Documentation of this anonymisation will be stored on a secure server at the Barts CVCTU, with unblinding for clinical reasons as necessary. The PI will be informed if this is necessary. Study analysis will occur in batches to prevent bias. For the assessment of LV volumes and mass, 2 readers will undertake assessment of inter and intra observer variation prior to analysis of blinded study images. Image analysis will be overseen and adjudicated by an experienced level 3 CMR reader (JCM).

End of study definition

The study will end after the final visit at week 16.

STATISTICAL ANALYSIS

In this study, we intend to recruit 80 patients in each treatment arm with stratification of 80 patients overall recruited to NITRATE-LVH and 80 recruited to the NITRATE-CBP arms (total n=160). This sample size will empower our trial to test for the primary and all major secondary endpoints listed below.

We determined the sample size using G*Power 3.0. Calculations were based on unpaired t-tests, a significance level of 0.05 (two-tailed) and the relevant standard deviations of the mean difference from published studies with reproducibility data: central systolic BP 1.7 mm Hg,[38] LV mass 9.9g,[39] CMR PWV 0.45 m/s,[40] Vicorder PWV 0.29 m/s.[40] For BP, we used measures of SD of systolic BP (as it has greater SD than diastolic BP) from the placebo limb of our previous intervention trial in hypertensive patients[13]: clinic systolic BP 8.4 mm Hg; ambulatory systolic BP 4.9 mm Hg; home systolic BP 3.9 mm Hg. International consensus documents on the optimal method for ultrasound FMD suggest using lab-specific data in sample size calculation and have suggested that 40 participants in a parallel study are sufficient to determine statistically important differences.[34] In a study of 4 week dietary nitrate intervention in hypertensive patients, SD of repeated measures of FMD in the placebo group was 0.6, whilst the mean difference between the groups was 1.0%[13], a SD of 1.1 .

Primary endpoints

For the individual stratified sub-groups:

NITRATE-LVH: For the primary endpoint of LV mass regression we have a power of 95% for detecting a 9g LV mass change (~6% change for a mildly hypertrophied heart of 150 g). Previously published data showed a 10% reduction in LV mass in hypertensives after 3 months treatment with spironolactone.[41] Furthermore, dietary advice aimed at BP reduction in the TOMHS study demonstrated mean 9/9 mm Hg BP reduction and ~20g LV mass reduction from baseline ~200g (by echocardiography) at 3 months post intervention, representing ~10% reduction in LV mass.[42]

NITRATE-CBP: For non-CMR PWV we have a power of > 95% for detecting a 0.6 m/s absolute change (previously demonstrated following 4 weeks dietary nitrate in hypertensive patients[13]). For CMR determined PWV we have a power of > 95% for detecting a 0.6 m/s absolute change.[13] For our primary end point of reduction in central systolic BP we expect a scaled reduction in BP relative to observed decreases in brachial BP (8.7 mm Hg clinic brachial systolic BP)[13] though evidence suggests that low-dose intravenous nitrite infusions act to selectively lower central BP in preference to brachial

BP and therefore expected differences may be larger.[43] Nevertheless, using our conservative estimate based on two-thirds brachial BP effect, we have > 90% power to detect a 5.8 mm Hg difference in central systolic BP.

In post hoc analyses data will be combined from the 2 groups to assess the overall impact of inorganic nitrate versus placebo on the entire cohort for BP, nitrate and nitrite analysis. In addition, correlation analyses will be conducted using Pearson's correlation between measures of NOx, LV mass, BP and PWV.

Secondary endpoints

a) Ultrasound FMD (%), > 90% power for detecting a 1.0% absolute change from a reduced FMD of ~5%.[13]

b) Brachial BP

i) Clinic > 85% power to detect 7 mm Hg absolute change.[13]

ii) Ambulatory > 95% power to detect 7 mm Hg absolute change.[13]

iii) Home > 85% power to detect 7 mm Hg absolute change.[13] The change observed in our 4 week study was ~7 mm Hg[13] but with reduced measurement frequency, the possibility of increase measurement variability and therefore SD was arbitrarily doubled for this calculation.

Our sample size calculations assume a drop-out rate of 15% (non-compliance with dietary intervention or follow-up visits) based on our centres previous observations.

Data will be analysed on an intention to treat basis. The data will be compared by multivariable regression analysis for each functional measure. The change from baseline in pre-specified primary, secondary and exploratory outcomes will be compared between groups by Student's t-test. We will adjust the results for multiple variables: sex, age, weight, body mass index and the initial differences between groups in LV mass.

Ethical considerations

The study protocol and any subsequent amendments, along with materials provided to participants and advertising material, was submitted to London - City and East REC. Written approval from the REC was obtained along with final sponsorship and NHS Health Research Authority (HRA) approval.

Safety considerations

The intervention is 70 mL of beetroot juice concentrate or nitrate-deplete beetroot juice (placebo; James White Drinks, UK). The nitrate is extracted using the same anion exchange technique to remove inorganic nitrate from the general drinking water supplies. There are no known serious side effects from these interventions and nitrate-free juice is classified as a foodstuff. In addition, several recent publications using the placebo juice are now available.[44–46] In the unlikely event of a serious adverse event (SAE) occurring directly as a result of the intervention, this would need to be reviewed by the chief investigator and the procedures followed as described below.

Safety reporting

An AE will be documented in the participants' medical notes and the case report form (CRF) and followed up by the investigators. Serious adverse events (SAEs) will be reported to the sponsor and REC where in the opinion of the chief investigator the event was either 'related' (i.e., resulted from administration of research procedures) and 'unexpected' (i.e., the type of event is not an expected occurrence).

SAEs considered to be 'related' and 'unexpected' will be recorded in the participants' notes, the sponsor SAE form and reported to the sponsor's joint research management office (JRMO) within 24 hours of the research team being notified, and to the main REC within 15 days. The co-investigators will be authorised to sign SAE forms in the absence of the PI. The intervention for the participant will be unblinded in the reporting of an 'unexpected and related' SAE, performed by an individual independent of the study procedures and will allow the investigators to remain blinded. The unblinding of single cases will only be performed if necessary for the safety of a participant.

MONITORING

Trial Steering Committee (TSC)

The TSC is composed of three independent experts in the fields of: cardiac MRI, hypertension and clinical trials along with the investigators and the data monitor and two lay members. This committee met before participant recruitment and will meet annually to assess safety, feasibility or any other arising problems and their recommendations followed.

Data Safety and Monitoring Board (DSMB)

The trial is classified as low-risk and does not require the formal setting up of a DSMB, however a DSMB has been established comprising a Clinical Trials Physician, Statistician and Cardiovascular

Physician. The DSMB met to review data after 20 patients in each study arm had completed their involvement, to advise about planned sample size and safety signals. The study will be subject to monitoring by the Sponsor, Queen Mary University of London, in accordance with their policies. Any monitoring findings will be relayed to the TSC by the Chief Investigator and acted in the best interest of patients, sponsor and funder.

Dissemination

The study will be performed in agreement with the Declaration of Helsinki and is approved by the Local REC (London – City and East; 10/H0703/98). Data collection will be completed by the third quarter of 2020. Primary and secondary analysis will start immediately after data collection is completed, with an aim to prepare publications for submission in 2021. The results of the trial will be published according to the CONSORT statement. Dissemination of results will be in peer-reviewed journal publications and presentations at national and international conferences. NITRATE-TOD is registered on Clinicaltrials.gov (NCT03088514).

Summary

This is the first randomised-controlled trial assessing the use of prolonged dietary nitrate treatment in hypertension-induced target organ damage, specifically LVH and arterial stiffness. This study will determine the potential of prolonged dietary nitrate as adjunctive therapy in patients with sub optimally controlled hypertension on anti-hypertensive medication.

Contributors: All authors listed above fulfil all three International Committee of Medical Journal Editors (ICMJE) guidelines for authorship, which are (1) substantial contributions to conception and design, acquisition of data or analysis, and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. CWZL was responsible for coordinating the contribution of all authors to this paper. VK, AA, JM and CWZL developed the protocol. CWZL was responsible for drafting this paper. KR, AM, JCM, VK and AA were responsible for editing and providing guidance on the paper. All authors were responsible for critically revising the paper. All authors approved the final version of this paper for submission. This study is supported by the CVCTU, a branch of the Barts CTU UKCRC Reg No. 4.

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Competing interests: AA is a co-director of Heartbeet Ltd, which is a start-up company that seeks to identify commercial potential of dietary nitrate.

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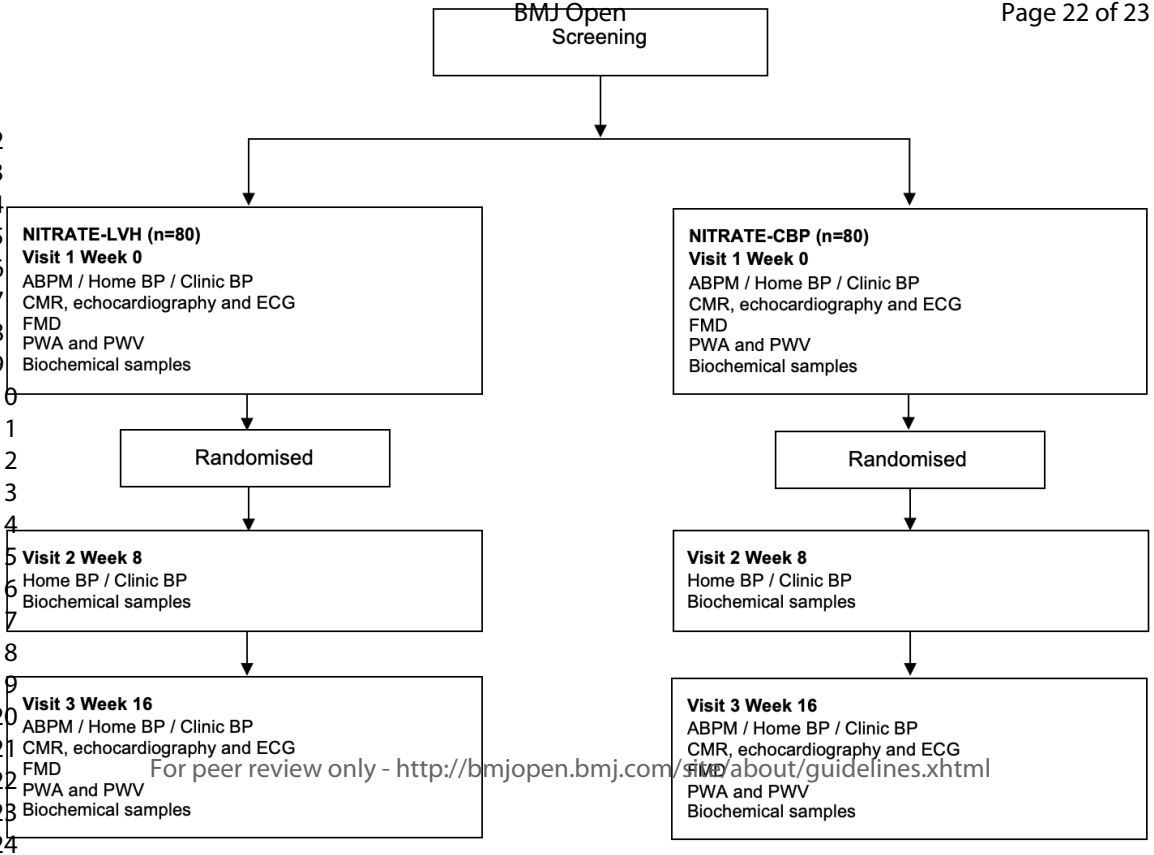

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	9
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	7
Participants	4a	Eligibility criteria for participants	7
	4b	Settings and locations where the data were collected	9
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	13
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	9
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	9
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	9
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	9
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	9

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	9
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	14
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	14
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	NA
	13b	For each group, losses and exclusions after randomisation, together with reasons	NA
Recruitment	14a	Dates defining the periods of recruitment and follow-up	NA
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	NA
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	NA
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	NA
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	NA
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	NA
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	NA
Other information			
Registration	23	Registration number and name of trial registry	16
Protocol	24	Where the full trial protocol can be accessed, if available	16
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL INVESTIGATING THE EFFECTS OF INORGANIC NITRATE IN HYPERTENSION-INDUCED TARGET ORGAN DAMAGE: PROTOCOL OF THE NITRATE-TOD STUDY IN THE UK

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RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL INVESTIGATING THE EFFECTS OF INORGANIC NITRATE IN HYPERTENSION-INDUCED TARGET ORGAN DAMAGE: PROTOCOL OF THE NITRATE-TOD STUDY IN THE UK

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ABSTRACT

Introduction

Arterial stiffness and left ventricular hypertrophy (LVH) are key markers of hypertensive target organ damage (TOD) associated with increased cardiovascular morbidity and mortality. We have previously shown that dietary inorganic nitrate supplementation lowers blood pressure (BP) in hypertension, however, whether this approach might also improve markers of hypertensive TOD is unknown. In this study, we will investigate whether daily dietary inorganic nitrate administration reduces left ventricular (LV) mass and improves measures of arterial stiffness.

Methods and Design

NITRATE-TOD is a double-blind, randomised, single-centre, placebo-controlled phase II trial aiming to enrol 160 patients with suboptimal BP control on 1 or more anti-hypertensives. Patients will be randomised to receive 4-month once daily dose of either nitrate-rich beetroot juice or nitrate-deplete beetroot juice (placebo). The primary outcomes are reduction in LV mass and reduction in pulse wave velocity (PWV) and central blood pressure (CBP).

The study has a power of 95% for detecting a 9g LV mass change by cardiovascular magnetic resonance (CMR) imaging (~6% change for a mildly hypertrophied heart of 150g). For PWV we have a power of > 95% for detecting a 0.6 m/s absolute change. For central systolic BP, we have a > 90% power to detect a 5.8 mm Hg difference in central systolic BP.

Secondary end-points include change in ultrasound flow mediated dilatation (FMD), change in plasma nitrate and nitrite concentration and change in BP.

Ethics and Dissemination

The study was approved by the London – City and East Research Ethics Committee (10/H0703/98). Trial results will be published according to the CONSORT statement and will be presented at conferences and reported in peer-reviewed journals.

ClinicalTrials.gov identifier: NCT03088514

Strengths and limitations of this study

- This is a randomised, placebo-controlled double-blind clinical trial assessing the potential of dietary nitrate in reducing both cardiac and vascular damage due to persistently elevated blood pressure.
- Dietary nitrate is a simple, easy to use and safe intervention that may be more acceptable to patients on long term medication for their blood pressure.
- This is a single-centre phase II study; therefore, the applicability to other centres is uncertain.

INTRODUCTION

Hypertension is the leading risk factor for cardiovascular diseases (CVD) worldwide.[1,2] In 2016, an estimated 17.9 million people died from CVDs, which represents 31% of all global deaths.[3] Approximately half of these deaths were caused by complications from hypertension.[4] Hence, novel and cost-effective therapeutic strategies continue to be sought. In this regard there has been a major emphasis to increase vegetable intake since increased consumption of a vegetable-rich diet particularly green leafy vegetables confers protection against CVD, including the lowering of BP[5,6]. One particular constituent of such vegetables that has been proposed to underlie the beneficial effects of this food group is inorganic nitrate; through bioconversion to nitrite, and then nitrite to nitric oxide (NO)[7] in the body. Importantly, provision of inorganic nitrate to hypertensive patients causes a rise in plasma nitrate and is associated with a decrease in BP.[8,9]

Dietary inorganic nitrate once ingested rapidly enters the circulation[8–10] with a proportion of this nitrate being extracted and then secreted and concentrated in the saliva.[11] Bacteria residing in the oral cavity convert this nitrate to nitrite which is then swallowed;[12] appearing soon after within the circulation. We have recently shown that daily supplementation with dietary nitrate (providing approximately 6.4 mmol nitrate daily) for 4 weeks was associated with robust, sustained and clinically meaningful reductions in BP (measured by clinic, ambulatory and home methods) of ~8/4 mm Hg.[13] However, persistent hypertension leads to both vascular and cardiac remodelling and whether inorganic nitrate-induced reductions in BP might be associated with improvements in either of these in the long term is unknown. Experimentally in patients one can assess this potential through the measurement of arterial stiffness and LV mass respectively, with increases of either conferring increased CV risk. Arterial stiffness (and its inverse measure, distensibility) refers to the decreased elasticity that develops in arteries as a consequence of vascular remodelling resulting from alterations of the fibrous components of the extracellular matrix (including elastin and collagen).[14] Importantly, increased arterial stiffness, as measured using carotid-femoral PWV,[15] is a strong predictor of CV events,[16] and there have been suggestions that drug development targeting this phenomenon is likely to provide a new genre of therapeutics in the combat against CVD.[17] Additionally, an increased central pulse pressure occurs when the large conduit blood vessels lose their elasticity and become less able to accommodate all of the blood ejected from the heart. This increase in pressure results in an increase in central systolic BP and causes an increase in the stress imposed on the left ventricle, which in turn can result in left ventricular hypertrophy (LVH).[18] Elevated LV mass is an initial compensatory mechanism to normalise wall stress due to elevated BP.

However, this cardiac remodelling eventually becomes maladaptive with an increase in myocardial oxygen consumption, cardiac chamber dilation, reduced LV contractility and progressive deterioration to heart failure.[19] The measure of LVH as a prognostic indicator of adverse outcome was first convincingly identified in the Framingham study,[20] and numerous studies since have confirmed the strong relationship between LVH and CV events and mortality.[21,22] Perhaps, more importantly, LV mass reduction is associated with further improvements in CV outcomes.[23,24]

It has been proposed that insufficient supply of endogenous NO likely contributes to the progression and worsening of arterial stiffness and LVH.[25] Pre-clinical studies have demonstrated that an elevation of systemic nitrite levels reduces cardiac hypertrophy in mice.[26] Moreover, administration of inorganic nitrate reduces PWV healthy volunteers and hypertensive patients in short term studies.[13,27] It is also possible that decreasing arterial stiffness improves ventricular-arterial coupling which might result in benefits in cardiac function.[28]

The objective of this study is to determine whether a dietary nitrate intervention might elevate the levels of NO in the body sufficiently to alter both heart and blood vessel remodelling in hypertensive patients with suboptimal BP control.

METHODOLOGY

Trial objectives

Aims of research: We wish to test the hypothesis that dietary supplementation of inorganic nitrate (in beetroot juice) elevates circulating nitrate and nitrite levels ultimately delivering NO to the vasculature and thereby improving endothelial, vascular and cardiac function in patients with treated yet uncontrolled hypertension. Specifically, the aim is to investigate whether prolonged dietary nitrate ingestion in hypertensive patients with suboptimal BP control, can cause reductions in LV mass and PWV.

Participant selection

This is a single-centre study, in which patients will be identified and recruited at Barts Health NHS Trust. In addition, other participant identification centres will be used to identify suitable participants via the NIHR Clinical Research Network and local NHS Trusts.

Original hypothesis

Dietary inorganic nitrate ingestion, in addition to existing pharmacological therapy, reduces BP leading to reductions in LV mass and arterial stiffness.

Primary end points

The primary end points are change in LV mass, determined using cardiovascular magnetic resonance imaging (CMR) (NITRATE-LVH arm) and a change in central systolic BP and PWV (NITRATE-CBP arm).

Secondary end points

Secondary endpoints are change in ultrasound determined brachial artery flow-mediated dilation (FMD), change in plasma nitrate and nitrite levels and a change in peripheral BP.

Exploratory end points

Exploratory end points for cardiac imaging include a change in aortic distensibility, LV systolic and diastolic function, LV volumes and ejection fraction, left atrial volumes and function and markers of myocardial fibrosis and oedema. We will assess changes in 12-lead ECG parameters of LVH. With regards to BP, we will assess for changes in BP variability, BP control rate and BP circadian pattern. We will also perform analysis of the salivary microbiome. Finally, we will assess changes in quality of life score (EQ-5D questionnaire) and biochemical measures e.g. urine albumin excretion, cholesterol fractions, B-natriuretic peptide (BNP) and troponin T.

Inclusion criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

1. Patients will be enrolled following an informed consent.
2. Aged 18-80 years.
3. The study subjects will be hypertensive with evidence of difficulty treating to target BP (daytime ABPM 135-170 and/or 85-105 mm Hg) on 1 or more antihypertensive agent, with insufficient efficacy or intolerance of medications.
4. For the NITRATE-LVH arm, echocardiographic evidence of LVH (LV mass indexed to body surface area (BSA); males > 115 g/m^2; females > 95 g/m^2).
5. Patients will have been established on an antihypertensive treatment regime for at least 1 month by the time of participation in the study and will not require changes in pharmacological intervention for the duration of the trial.

Exclusion criteria

Unless specified, a subject will not be eligible for inclusion in this study if any of the following criteria apply:

1. History of chronic viral hepatitis (including presence of hepatitis B surface antigen or hepatitis C antibody), or other chronic hepatic disorders.
2. History of increased liver function tests (ALT, AST) due to acute or chronic liver conditions, 3x above the upper limit of normal or bilirubin 1.5x above the upper limit of normal at screening.
3. Renal impairment with creatinine clearance (eGFR) of < 50 ml/min at screening.
4. Patients with diabetes mellitus, defined by previous history of diabetes or HbA1c > 6.5% (> 48 mmol/mol) at screening.
5. Subjects with LDLc, > 7.5 mmol/l and/or triglyceride level > 10 mmol/l.
6. History of heart failure defined as NYHA class II - IV or those with known LV dysfunction (LV ejection fraction < 40%) regardless of symptomatic status.
7. History of malignancy within the past 5 years, other than non-melanoma skin cancer.
8. Current life-threatening condition other than vascular disease (e.g. very severe chronic airways disease, HIV positive, life-threatening arrhythmias) that may prevent a subject from completing the study.

9. Use of an investigational device or investigational drug within 30 days or 5 half-lives (whichever is longer) preceding the first dose of study medication.
10. Subjects who will commence or who are likely to commence regular treatment with non-steroidal anti-inflammatory drugs (NSAIDs) (other than aspirin), from screening until study completion.
11. Any non-stable dosing of ongoing medication regimens throughout the study trial.
12. Drug abuse within the past 6 months.
13. The subject has a three-month prior history of regular alcohol consumption exceeding an average weekly intake of > 28 units (or an average daily intake of greater than 3 units) for males, or an average weekly intake of > 21 units (or an average daily intake of greater than 2 units) for females. 1 unit is equivalent to a half-pint (284mL) of beer/lager; 25mL measure of spirits or 125mL of wine.
14. Any other subject whom the Investigator deems unsuitable for the study (e.g. due to other medical reasons, laboratory abnormalities, expected study medication noncompliance, or subject's unwillingness to comply with all study-related study procedures).
15. Subjects with rheumatoid arthritis, connective tissue disorders and other conditions known to be associated with chronic inflammation (e.g. Inflammatory Bowel Disease).
16. Subjects with any acute infection, or recent systemic (oral or IV) antibiotics within 1 month of screening, or significant trauma (burns, fractures).
17. Subjects who have donated more than 500 mL of blood within 56 days prior to the study medication administration.
18. Self-reported use of anti-microbial mouthwash or tongue scrapes.
19. Concomitant xanthine oxidase inhibitors (such as allopurinol).
20. Known history of significant claustrophobia, previous intolerance of CMR imaging or known (or suspected) incompatible metallic implant.
21. Pregnancy.
22. Allergy to gadolinium-based contrast agents used for CMR.
23. Patients with known LVH caused by another established pathology diagnosed prior to or at screening e.g. severe aortic stenosis, hypertrophic cardiomyopathy, amyloidosis and Fabry disease.

Exceptions to the exclusion criteria:

- For criteria 18, patients can enter the trial if they discontinue the use of anti-microbial mouthwash for the duration of the clinical trial.

- Criteria 20 and 22 do not apply to participants who will not have a CMR scan in the NITRATE-CBP arm.

Study design and intervention

This is a prospective double-blind, placebo-controlled, clinical study. A total of 160 patients (male and female, age 18–80) with hypertension as per requirements indicated above will be recruited. Figure 1 shows a summary of the study scheme. Patients will be stratified at screening into 2 arms. Participants with LVH at screening echocardiography will enter the NITRATE-LVH arm (n=80) whilst those who do not have LVH but satisfy the other eligibility criteria will enter the NITRATE-CBP arm (n=80). LVH is defined by increased LV mass determined by acquiring a parasternal long axis image and subsequently measured for the interventricular septal wall thickness, end-diastolic LV cavity diameter and posterior LV wall thickness according to published guidelines.[29] LV mass is calculated as previously described and indexed to body surface area (BSA).[30]

All visits will take place at the William Harvey Heart Centre, Queen Mary University of London and Barts Heart Centre, St Bartholomew's Hospital, Barts Health NHS Trust. Patients will be randomised to receive 70 mL of a beetroot juice concentrate containing ~6 mmol nitrate or nitrate-depleted placebo juice concentrate (James White Drinks, UK) control. The volunteers will start taking their daily dose after completion of their baseline visit (visit 1) for 16 weeks. Patients will be advised to take their dose of juice at the same time each day, preferably in the morning with their breakfast. Patients will be provided with dietary advice in relation to the calorific content of interventions (both ~100kcal). In addition to baseline and 16 week visits, patients will return to the clinic at 8 weeks for collection of samples for NOx analysis and for discussion of any adherence issues.

Randomisation and blinding process

Patients will be block randomised on a 1:1 basis to receive either dietary nitrate or placebo, using a binary random number sequence (http://www.random.org). Treatment assignment for volunteers in the dietary nitrate and placebo groups will remain blinded until data lock and statistical analysis at the end of the study. If unblinding is required, the chief investigator for the study will be informed. A list of the unblinded treatments will be kept in a secure location at the Barts Cardiovascular Clinical Trials Unit (CVCTU). The unblinding procedure will be available at all times.

Patient and public involvement

Patients were directly involved in the design and planning of the study. Twenty previous Barts Health NHS Trust patients were surveyed regarding aspects of trials involving dietary interventions, which informed this protocol.

Study start and end dates

Recruitment commenced on 23rd March 2017. The provisional end date for recruitment is September 2020.

Methods to be used

Blood, saliva and urine analysis

In this study, venous blood will be taken using a 21-gauge butterfly needle at each visit. Blood samples will be centrifuged immediately and plasma separated. The plasma samples will be snap frozen in liquid nitrogen and stored at -80°C. These samples will be used for the purposes of making biochemical measurements and will be discarded once used. Similarly, saliva and urine will be collected at each visit. Saliva will be centrifuged, and a pellet generated. This pellet contains oral bacteria that have dislodged from the oral cavity. This pellet will be frozen for later analysis of oral bacteria by second-generation genome sequencing.

Nitrate and nitrite levels in saliva, blood and urine samples will be determined using ozone chemiluminescence as previously described.[31,32] In brief, total nitrate and nitrite concentration (termed 'NOx') will be determined by adding samples to 0.1mol/L vanadium (III) chloride in 1M hydrochloric acid refluxing at 95°C under nitrogen. Nitrite concentration will be determined by the addition of samples to 0.09 mol/L potassium iodide in glacial acetic acid under nitrogen at room temperature. Nitrate concentration will be calculated by the subtraction of [nitrite] from [NOx]. All measurements will be conducted by an individual blinded to the intervention. In addition, a sample from each batch of juice sent to each volunteer will be analysed for NOx content.

Pulse wave analysis and pulse wave velocity

Pulse wave analysis (PWA) and PWV are measures of arterial stiffness, which will be determined by a non-invasive Vicorder device (Skidmore Medical Limited, Bristol, UK). For PWA, the pulse wave will be recorded from the brachial cuff applied to the non-dominant arm. For PWV, the pulse wave will be simultaneously recorded from the carotid and femoral site using an oscillometric method. A small, inflatable neck pad will be placed directly over a carotid artery and secured around the neck by a Velcro tab and a cuff will also be placed around the patient's ipsilateral upper thigh. Both carotid and

femoral cuffs will be simultaneously inflated automatically to 65 mm Hg and the corresponding oscillometric signal from each cuff digitally analysed to extract the pulse time delay. To estimate the aortic length, the distance between the sternal notch and the thigh cuff will be measured. From these measurements, PWV can be derived as PWV = aortic distance/pulse time delay.[33]

Flow-mediated dilation

FMD will be used to non-invasively assess endothelial function, using vascular ultrasound to measure the increase in brachial artery diameter in response to increased flow[34] as previously described.[35] A high-resolution external vascular ultrasound Siemens/Acuson Sequoia C256 Colour Doppler with a 7.0-MHz linear-array transducer supported by a stereotactic clamp will be used to assess the vessel diameter in the right arm. The vessel will be scanned in longitudinal section and the centre will be identified when the clearest views of the anterior and posterior artery walls have been obtained. Images will be magnified with a resolution box function and images of the brachial artery acquired continuously using semi-automated edge detection software (FMD Studio, Quipu s.r.l, Pisa, Italy) and analysed in real time. An automatic mathematical contour tracking operator locates the pulsed-wave Doppler of the brachial artery which will be used to measure the diameter and blood flow velocity continuously for 1 minute at baseline, then during 5 minutes of reduced blood flow (induced by inflation to 300 mm Hg of a pneumatic cuff placed on the right forearm site distal to the segment of artery being analysed), and finally for a further 5 minutes during reactive hyperaemia following cuff release. FMD is defined as the maximum percentage increase in vessel diameter during reactive hyperaemia. This procedure will be performed at visit 1 (baseline) and visit 3 (16 weeks). In some volunteers, following FMD, 0.4 mg of sublingual GTN (glyceryl trinitrate) will be administered at visit 1 and visit 3 to determine whether changes in FMD responses following intervention might be due to changes in smooth muscle reactivity.

Transthoracic echocardiography

At visit 1 and 3, echocardiography is also performed to assess LV diastolic function. Images will be acquired for measures of LV diastolic function including left atrial end-diastolic volumes, pulmonary vein s/d ratio, mitral valve E/A ratio and E/e'.[29,36] Images will be acquired and analysed by an operator accredited by the British Society of Echocardiography.

CMR

The most accurate and reproducible measurements of LVH are made by CMR.[37] At visits 1 and 3, CMR in the NITRATE-LVH arm and those that consent in the NITRATE-CBP arm will be used to assess

LV mass, volumes and ejection fraction, T1 and T2 mapping, patterns of late gadolinium enhancement (LGE), aortic distensibility and PWV. Participants will have a peripheral venous cannula inserted and gadolinium injected at doses up to 0.2 mmol/kg.

To allow a thorough assessment of cardiac function, the following imaging sequences will be undertaken:

- Scout images, including black and white blood transverse slices of the thorax
- Long axis cines (4-chamber, 2-chamber, LVOT-1, LVOT-2, aortic valve short axis)
- Short axis cine stack
- T1 and T2 mapping
- LGE in the 4-chamber, 2-chamber, 3-chamber views and short axis stack
- Post contrast T1 mapping for extra-cellular volume (ECV) quantification
- Sagittal aortic views and aortic flow sequences for assessment of aortic distensibility and PWV.

Short axis images will be acquired using standard sequences i.e. 10 – 12 slices of 8mm thickness with 2mm gap to achieve whole LV coverage. This is undertaken with the patient in held expiration. Cine and LGE short axis stacks will be acquired using identical scanning geometry to assure correct image registration.

The following offline parameters will be measured:

- Presence of significant extra cardiac pathology
- Left ventricular ejection fraction, volumes and mass
- Distribution of LGE
- Analysis of pre and post contrast T1 mapping and T2 mapping. Short axis images will be segmented according to the AHA model as previously described[38]
- Aortic distensibility and pulse wave velocity.

For the analysis of study outcomes, each study will be anonymised by a third party. This will be separate from the study ID to avoid bias between pre- and post-treatment studies. Documentation of this anonymisation will be stored on a secure server at the Barts CVCTU, with unblinding for clinical reasons as necessary. The PI will be informed if this is necessary. Study analysis will occur in batches to prevent bias. For the assessment of LV volumes and mass, 2 readers will undertake assessment of

inter and intra observer variation prior to analysis of blinded study images. Image analysis will be overseen and adjudicated by an experienced level 3 CMR reader (JCM).

End of study definition

The study will end after the final visit at week 16.

STATISTICAL ANALYSIS

In this study, we intend to recruit 80 patients in each treatment arm with stratification of 80 patients overall recruited to NITRATE-LVH and 80 recruited to the NITRATE-CBP arms (total n=160). This sample size will empower our trial to test for the primary and all major secondary endpoints listed below.

We determined the sample size using G*Power 3.0. Calculations were based on unpaired t-tests, a significance level of 0.05 (two-tailed) and the relevant standard deviations of the mean difference from published studies with reproducibility data: central systolic BP 1.7 mm Hg,[39] LV mass 9.9g,[40] CMR PWV 0.45 m/s,[41] Vicorder PWV 0.29 m/s.[41] For BP, we used measures of SD of systolic BP (as it has greater SD than diastolic BP) from the placebo limb of our previous intervention trial in hypertensive patients[13]: clinic systolic BP 8.4 mm Hg; ambulatory systolic BP 4.9 mm Hg; home systolic BP 3.9 mm Hg. International consensus documents on the optimal method for ultrasound FMD suggest using lab-specific data in sample size calculation and have suggested that 40 participants in a parallel study are sufficient to determine statistically important differences.[35] In a study of 4 week dietary nitrate intervention in hypertensive patients, SD of repeated measures of FMD in the placebo group was 0.6, whilst the mean difference between the groups was 1.0%[13], a SD of 1.1 .

Primary endpoints

For the individual stratified sub-groups:

NITRATE-LVH: For the primary endpoint of LV mass regression we have a power of 95% for detecting a 9g LV mass change (~6% change for a mildly hypertrophied heart of 150 g). Previously published data showed a 10% reduction in LV mass in hypertensives after 3 months treatment with spironolactone.[42] Furthermore, dietary advice aimed at BP reduction in the TOMHS study demonstrated mean 9/9 mm Hg BP reduction and ~20g LV mass reduction from baseline ~200g (by echocardiography) at 3 months post intervention, representing ~10% reduction in LV mass.[43]

NITRATE-CBP: For non-CMR PWV we have a power of > 95% for detecting a 0.6 m/s absolute change (previously demonstrated following 4 weeks dietary nitrate in hypertensive patients[13]). For CMR determined PWV we have a power of > 95% for detecting a 0.6 m/s absolute change.[13] For our primary end point of reduction in central systolic BP we expect a scaled reduction in BP relative to observed decreases in brachial BP (8.7 mm Hg clinic brachial systolic BP)[13] though evidence suggests that low-dose intravenous nitrite infusions act to selectively lower central BP in preference to brachial BP and therefore expected differences may be larger.[44] Nevertheless, using our conservative estimate based on two-thirds brachial BP effect, we have > 90% power to detect a 5.8 mm Hg difference in central systolic BP.

In post hoc analyses data will be combined from the 2 groups to assess the overall impact of inorganic nitrate versus placebo on the entire cohort for BP, nitrate and nitrite analysis. In addition, correlation analyses will be conducted using Pearson's correlation between measures of NOx, LV mass, BP and PWV.

Secondary endpoints

a) Ultrasound FMD (%), > 90% power for detecting a 1.0% absolute change from a reduced FMD of ~5%.[13]

b) Brachial BP

i) Clinic > 85% power to detect 7 mm Hg absolute change.[13]

ii) Ambulatory > 95% power to detect 7 mm Hg absolute change.[13]

iii) Home > 85% power to detect 7 mm Hg absolute change.[13] The change observed in our 4 week study was ~7 mm Hg[13] but with reduced measurement frequency, the possibility of increase measurement variability and therefore SD was arbitrarily doubled for this calculation.

Our sample size calculations assume a drop-out rate of 15% (non-compliance with dietary intervention or follow-up visits) based on our centres previous observations.

Data will be analysed on an intention to treat basis. The data will be compared by multivariable regression analysis for each functional measure. The change from baseline in pre-specified primary, secondary and exploratory outcomes will be compared between groups by Student's t-test. We will adjust the results for multiple variables: sex, age, weight, body mass index and the initial differences between groups in LV mass.

Ethical considerations

The NIHR Barts Biomedical Research Centre Patient and Public Advisory Group reviewed this protocol prior to submission to the Research Ethics Committee (REC). The study protocol and any subsequent amendments, along with materials provided to participants and advertising material, was submitted to London - City and East REC. Written approval from the REC was obtained along with final sponsorship and NHS Health Research Authority (HRA) approval.

Safety considerations

The intervention is 70 mL of beetroot juice concentrate or nitrate-deplete beetroot juice (placebo; James White Drinks, UK). The nitrate is extracted using the same anion exchange technique to remove inorganic nitrate from the general drinking water supplies. There are no known serious side effects from these interventions and nitrate-free juice is classified as a foodstuff. In addition, several recent publications using the placebo juice are now available.[45–47] In the unlikely event of a serious adverse event (SAE) occurring directly as a result of the intervention, this would need to be reviewed by the chief investigator and the procedures followed as described below.

Safety reporting

An AE will be documented in the participants' medical notes and the case report form (CRF) and followed up by the investigators. Serious adverse events (SAEs) will be reported to the sponsor and REC where in the opinion of the chief investigator the event was either 'related' (i.e., resulted from administration of research procedures) and 'unexpected' (i.e., the type of event is not an expected occurrence).

SAEs considered to be 'related' and 'unexpected' will be recorded in the participants' notes, the sponsor SAE form and reported to the sponsor's joint research management office (JRMO) within 24 hours of the research team being notified, and to the main REC within 15 days. The co-investigators will be authorised to sign SAE forms in the absence of the PI. The intervention for the participant will be unblinded in the reporting of an 'unexpected and related' SAE, performed by an individual independent of the study procedures and will allow the investigators to remain blinded. The unblinding of single cases will only be performed if necessary for the safety of a participant.

MONITORING

Trial Steering Committee (TSC)

The TSC is composed of three independent experts in the fields of: cardiac MRI, hypertension and clinical trials along with the investigators and the data monitor and two lay members. This committee met before participant recruitment and will meet annually to assess safety, feasibility or any other arising problems and their recommendations followed.

Data Safety and Monitoring Board (DSMB)

The trial is classified as low-risk and does not require the formal setting up of a DSMB, however a DSMB has been established comprising a Clinical Trials Physician, Statistician and Cardiovascular Physician. The DSMB met to review data after 20 patients in each study arm had completed their involvement, to advise about planned sample size and safety signals. The study will be subject to monitoring by the Sponsor, Queen Mary University of London, in accordance with their policies. Any monitoring findings will be relayed to the TSC by the Chief Investigator and acted in the best interest of patients, sponsor and funder.

Dissemination

The study will be performed in agreement with the Declaration of Helsinki and is approved by the Local REC (London – City and East; 10/H0703/98). Data collection will be completed by the third quarter of 2020. Primary and secondary analysis will start immediately after data collection is completed, with an aim to prepare publications for submission in 2021. The results of the trial will be published according to the CONSORT statement. Dissemination of results will be in peer-reviewed journal publications and presentations at national and international conferences. NITRATE-TOD is registered on Clinicaltrials.gov (NCT03088514).

Summary

This is the first randomised-controlled trial assessing the use of prolonged dietary nitrate treatment in hypertension-induced target organ damage, specifically LVH and arterial stiffness. This study will determine the potential of prolonged dietary nitrate as adjunctive therapy in patients with sub optimally controlled hypertension on anti-hypertensive medication.

Contributors: All authors listed above fulfil all three International Committee of Medical Journal Editors (ICMJE) guidelines for authorship, which are (1) substantial contributions to conception and design, acquisition of data or analysis, and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. AA raised the funding. AA and VK designed the study. CWZL was responsible for coordinating the

contribution of all authors to this paper. AA, VK, JM and CWZL developed the protocol. CWZL and AJPH are involved the acquisition of data or data analysis. CWZL, KR, JC, AS and CP were responsible for drafting this paper. AM, CD, JM, VK and AA were responsible for editing and providing guidance on the paper. All authors were responsible for critically revising the paper. All authors approved the final version of this paper for submission. This study is supported by the CVCTU, a branch of the Barts CTU UKCRC Reg No. 4.

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Competing interests: AA is a co-director of Heartbeet Ltd, which is a start-up company that seeks to identify commercial potential of dietary nitrate.

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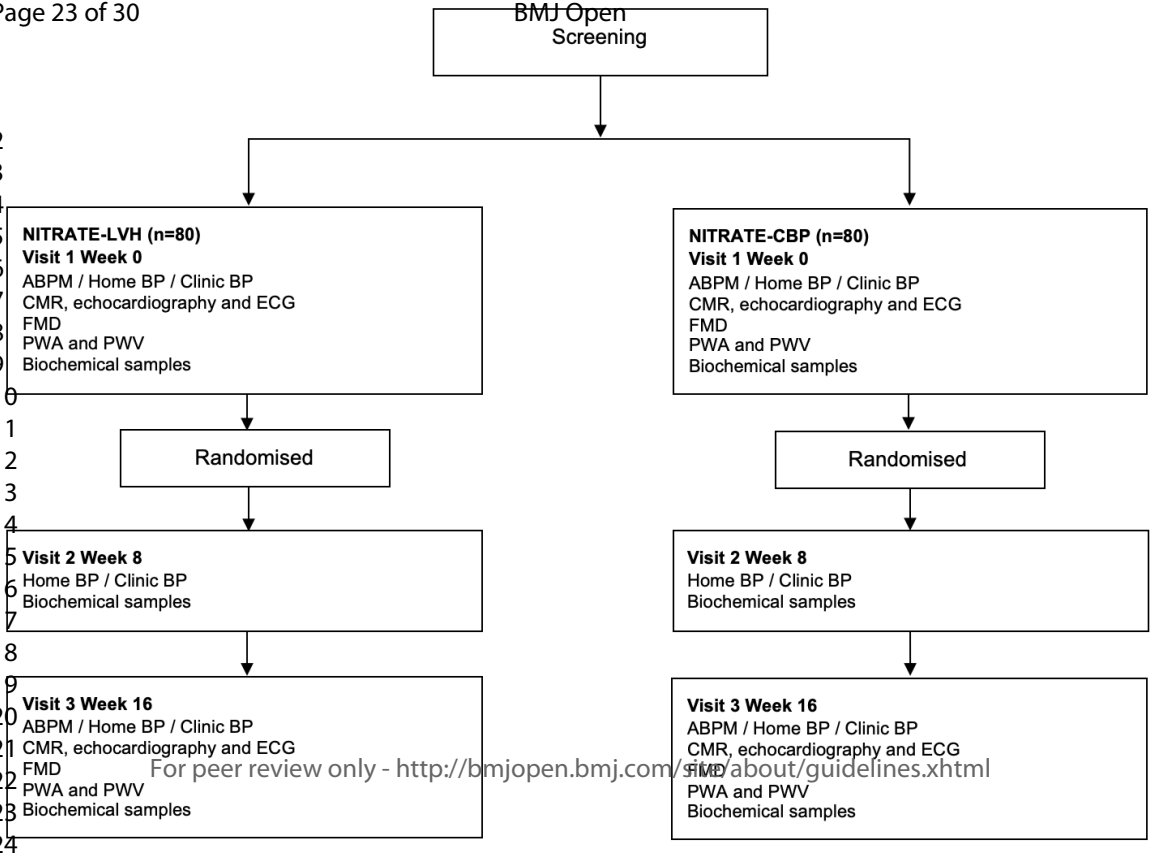
Figure 1 Legend

Figure 1. Study protocol. ABPM, ambulatory blood pressure monitor; ECG, electrocardiogram; CMR, cardiovascular magnetic resonance imaging; FMD, flow mediated dilation; PWA, pulse wave analysis; PWV, pulse wave velocity.



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page	Line no.
Administrative information				
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2	31
	2b	All items from the World Health Organization Trial Registration Data Set	In full protocol	
Protocol version	3	Date and version identifier	In full protocol	
Funding	4	Sources and types of financial, material, and other support	17	9
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1	5
	5b	Name and contact information for the trial sponsor	1	16
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	In full protocol	

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	16	1
Introduction				
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4	78
	6b	Explanation for choice of comparators	5	15
Objectives	7	Specific objectives or hypotheses	6	2
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9	5
Methods: Participants, interventions, and outcomes				
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9	15
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7	2
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9	16
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	15	32

	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9	22
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA	NA
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6	19
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1	
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13	11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6	10
Methods: Assignment of interventions (for controlled trials)				
Allocation:				
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9	25
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9	25
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	In full protocol	

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9	5
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9	25
Methods: Data collection, management, and analysis				
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10	11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14	32
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	In full protocol	
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13	9
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13	9
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13	9
Methods: Monitoring				

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	16	9
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	In full protocol	
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15	20
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	In full protocol	
Ethics and dissemination				
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	15	4
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	In full protocol	
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	In full protocol	
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	In full protocol	

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	17	11
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	In full protocol	
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	In full protocol	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16	18
	31b	Authorship eligibility guidelines and any intended use of professional writers	16	33
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA	
Appendices				
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	In Supplement	
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	10	11

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	9
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	7
Participants	4a	Eligibility criteria for participants	7
	4b	Settings and locations where the data were collected	9
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	13
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	9
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	9
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	9
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	9
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	9

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	9
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	14
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	14
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	NA
	13b	For each group, losses and exclusions after randomisation, together with reasons	NA
Recruitment	14a	Dates defining the periods of recruitment and follow-up	NA
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	NA
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	NA
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	NA
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	NA
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	NA
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	NA
Other information			
Registration	23	Registration number and name of trial registry	16
Protocol	24	Where the full trial protocol can be accessed, if available	16
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	17

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL INVESTIGATING THE EFFECTS OF INORGANIC NITRATE IN HYPERTENSION-INDUCED TARGET ORGAN DAMAGE: PROTOCOL OF THE NITRATE-TOD STUDY IN THE UK

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RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL INVESTIGATING THE EFFECTS OF INORGANIC NITRATE IN HYPERTENSION-INDUCED TARGET ORGAN DAMAGE: PROTOCOL OF THE NITRATE-TOD STUDY IN THE UK

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ABSTRACT

Introduction

Arterial stiffness and left ventricular hypertrophy (LVH) are key markers of hypertensive target organ damage (TOD) associated with increased cardiovascular morbidity and mortality. We have previously shown that dietary inorganic nitrate supplementation lowers blood pressure (BP) in hypertension, however, whether this approach might also improve markers of hypertensive TOD is unknown. In this study, we will investigate whether daily dietary inorganic nitrate administration reduces left ventricular (LV) mass and improves measures of arterial stiffness.

Methods and Design

NITRATE-TOD is a double-blind, randomised, single-centre, placebo-controlled phase II trial aiming to enrol 160 patients with suboptimal BP control on 1 or more anti-hypertensives. Patients will be randomised to receive 4-month once daily dose of either nitrate-rich beetroot juice or nitrate-deplete beetroot juice (placebo). The primary outcomes are reduction in LV mass and reduction in pulse wave velocity (PWV) and central blood pressure (CBP).

The study has a power of 95% for detecting a 9g LV mass change by cardiovascular magnetic resonance (CMR) imaging (~6% change for a mildly hypertrophied heart of 150g). For PWV we have a power of > 95% for detecting a 0.6 m/s absolute change. For central systolic BP, we have a > 90% power to detect a 5.8 mm Hg difference in central systolic BP.

Secondary end-points include change in ultrasound flow mediated dilatation (FMD), change in plasma nitrate and nitrite concentration and change in BP.

Ethics and Dissemination

The study was approved by the London – City and East Research Ethics Committee (10/H0703/98). Trial results will be published according to the CONSORT statement and will be presented at conferences and reported in peer-reviewed journals.

ClinicalTrials.gov identifier: NCT03088514

Strengths and limitations of this study

- This is a randomised, placebo-controlled double-blind clinical trial assessing the potential of dietary nitrate in reducing both cardiac and vascular damage due to persistently elevated blood pressure.
- Dietary nitrate is a simple, easy to use and safe intervention that may be more acceptable to patients on long term medication for their blood pressure.
- This is a single-centre phase II study; therefore, the applicability to other centres is uncertain.

INTRODUCTION

Hypertension is the leading risk factor for cardiovascular diseases (CVD) worldwide.[1,2] In 2016, an estimated 17.9 million people died from CVDs, which represents 31% of all global deaths.[3] Approximately half of these deaths were caused by complications from hypertension.[4] Hence, novel and cost-effective therapeutic strategies continue to be sought. In this regard there has been a major emphasis to increase vegetable intake since increased consumption of a vegetable-rich diet particularly green leafy vegetables confers protection against CVD, including the lowering of BP[5,6]. One particular constituent of such vegetables that has been proposed to underlie the beneficial effects of this food group is inorganic nitrate; through bioconversion to nitrite, and then nitrite to nitric oxide (NO)[7] in the body. Importantly, provision of inorganic nitrate to hypertensive patients causes a rise in plasma nitrate and is associated with a decrease in BP.[8,9]

Dietary inorganic nitrate once ingested rapidly enters the circulation[8–10] with a proportion of this nitrate being extracted and then secreted and concentrated in the saliva.[11] Bacteria residing in the oral cavity convert this nitrate to nitrite which is then swallowed;[12] appearing soon after within the circulation. We have recently shown that daily supplementation with dietary nitrate (providing approximately 6.4 mmol nitrate daily) for 4 weeks was associated with robust, sustained and clinically meaningful reductions in BP (measured by clinic, ambulatory and home methods) of ~8/4 mm Hg.[13] However, persistent hypertension leads to both vascular and cardiac remodelling and whether inorganic nitrate-induced reductions in BP might be associated with improvements in either of these in the long term is unknown. Experimentally in patients one can assess this potential through the measurement of arterial stiffness and LV mass respectively, with increases of either conferring increased CV risk. Arterial stiffness (and its inverse measure, distensibility) refers to the decreased elasticity that develops in arteries as a consequence of vascular remodelling resulting from alterations of the fibrous components of the extracellular matrix (including elastin and collagen).[14] Importantly, increased arterial stiffness, as measured using carotid-femoral PWV,[15] is a strong predictor of CV events,[16] and there have been suggestions that drug development targeting this phenomenon is likely to provide a new genre of therapeutics in the combat against CVD.[17] Additionally, an increased central pulse pressure occurs when the large conduit blood vessels lose their elasticity and become less able to accommodate all of the blood ejected from the heart. This increase in pressure results in an increase in central systolic BP and causes an increase in the stress imposed on the left ventricle, which in turn can result in left ventricular hypertrophy (LVH).[18] Elevated LV mass is an initial compensatory mechanism to normalise wall stress due to elevated BP.

However, this cardiac remodelling eventually becomes maladaptive with an increase in myocardial oxygen consumption, cardiac chamber dilation, reduced LV contractility and progressive deterioration to heart failure.[19] The measure of LVH as a prognostic indicator of adverse outcome was first convincingly identified in the Framingham study,[20] and numerous studies since have confirmed the strong relationship between LVH and CV events and mortality.[21,22] Perhaps, more importantly, LV mass reduction is associated with further improvements in CV outcomes.[23,24]

It has been proposed that insufficient supply of endogenous NO likely contributes to the progression and worsening of arterial stiffness and LVH.[25] Pre-clinical studies have demonstrated that an elevation of systemic nitrite levels reduces cardiac hypertrophy in mice.[26] Moreover, administration of inorganic nitrate reduces PWV healthy volunteers and hypertensive patients in short term studies.[13,27] It is also possible that decreasing arterial stiffness improves ventricular-arterial coupling which might result in benefits in cardiac function.[28]

The objective of this study is to determine whether a dietary nitrate intervention might elevate the levels of NO in the body sufficiently to alter both heart and blood vessel remodelling in hypertensive patients with suboptimal BP control.

METHODOLOGY

Trial objectives

Aims of research: We wish to test the hypothesis that dietary supplementation of inorganic nitrate (in beetroot juice) elevates circulating nitrate and nitrite levels ultimately delivering NO to the vasculature and thereby improving endothelial, vascular and cardiac function in patients with treated yet uncontrolled hypertension. Specifically, the aim is to investigate whether prolonged dietary nitrate ingestion in hypertensive patients with suboptimal BP control, can cause reductions in LV mass and PWV.

Participant selection

This is a single-centre study, in which patients will be identified and recruited at Barts Health NHS Trust. In addition, other participant identification centres will be used to identify suitable participants via the NIHR Clinical Research Network and local NHS Trusts.

Original hypothesis

Dietary inorganic nitrate ingestion, in addition to existing pharmacological therapy, reduces BP leading to reductions in LV mass and arterial stiffness.

Primary end points

The primary end points are change in LV mass, determined using cardiovascular magnetic resonance imaging (CMR) (NITRATE-LVH arm) and a change in central systolic BP and PWV (NITRATE-CBP arm).

Secondary end points

Secondary endpoints are change in ultrasound determined brachial artery flow-mediated dilation (FMD), change in plasma nitrate and nitrite levels and a change in peripheral BP.

Exploratory end points

Exploratory end points for cardiac imaging include a change in aortic distensibility, LV systolic and diastolic function, LV volumes and ejection fraction, left atrial volumes and function and markers of myocardial fibrosis and oedema. We will assess changes in 12-lead ECG parameters of LVH. With regards to BP, we will assess for changes in BP variability, BP control rate and BP circadian pattern. We will also perform analysis of the salivary microbiome. Finally, we will assess changes in quality of life score (EQ-5D questionnaire) and biochemical measures e.g. urine albumin excretion, cholesterol fractions, B-natriuretic peptide (BNP) and troponin T.

Inclusion criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

1. Patients will be enrolled following an informed consent.
2. Aged 18-80 years.
3. The study subjects will be hypertensive with evidence of difficulty treating to target BP (daytime ABPM 135-170 and/or 85-105 mm Hg) on 1 or more antihypertensive agent, with insufficient efficacy or intolerance of medications.
4. For the NITRATE-LVH arm, echocardiographic evidence of LVH (LV mass indexed to body surface area (BSA); males > 115 g/m^2; females > 95 g/m^2).
5. Patients will have been established on an antihypertensive treatment regime for at least 1 month by the time of participation in the study and will not require changes in pharmacological intervention for the duration of the trial.

Exclusion criteria

Unless specified, a subject will not be eligible for inclusion in this study if any of the following criteria apply:

1. History of chronic viral hepatitis (including presence of hepatitis B surface antigen or hepatitis C antibody), or other chronic hepatic disorders.
2. History of increased liver function tests (ALT, AST) due to acute or chronic liver conditions, 3x above the upper limit of normal or bilirubin 1.5x above the upper limit of normal at screening.
3. Renal impairment with creatinine clearance (eGFR) of < 50 ml/min at screening.
4. Patients with diabetes mellitus, defined by previous history of diabetes or HbA1c > 6.5% (> 48 mmol/mol) at screening.
5. Subjects with LDLc, > 7.5 mmol/l and/or triglyceride level > 10 mmol/l.
6. History of heart failure defined as NYHA class II - IV or those with known LV dysfunction (LV ejection fraction < 40%) regardless of symptomatic status.
7. History of malignancy within the past 5 years, other than non-melanoma skin cancer.
8. Current life-threatening condition other than vascular disease (e.g. very severe chronic airways disease, HIV positive, life-threatening arrhythmias) that may prevent a subject from completing the study.

9. Use of an investigational device or investigational drug within 30 days or 5 half-lives (whichever is longer) preceding the first dose of study medication.
10. Subjects who will commence or who are likely to commence regular treatment with non-steroidal anti-inflammatory drugs (NSAIDs) (other than aspirin), from screening until study completion.
11. Any non-stable dosing of ongoing medication regimens throughout the study trial.
12. Drug abuse within the past 6 months.
13. The subject has a three-month prior history of regular alcohol consumption exceeding an average weekly intake of > 28 units (or an average daily intake of greater than 3 units) for males, or an average weekly intake of > 21 units (or an average daily intake of greater than 2 units) for females. 1 unit is equivalent to a half-pint (284mL) of beer/lager; 25mL measure of spirits or 125mL of wine.
14. Any other subject whom the Investigator deems unsuitable for the study (e.g. due to other medical reasons, laboratory abnormalities, expected study medication noncompliance, or subject's unwillingness to comply with all study-related study procedures).
15. Subjects with rheumatoid arthritis, connective tissue disorders and other conditions known to be associated with chronic inflammation (e.g. Inflammatory Bowel Disease).
16. Subjects with any acute infection, or recent systemic (oral or IV) antibiotics within 1 month of screening, or significant trauma (burns, fractures).
17. Subjects who have donated more than 500 mL of blood within 56 days prior to the study medication administration.
18. Self-reported use of anti-microbial mouthwash or tongue scrapes.
19. Concomitant xanthine oxidase inhibitors (such as allopurinol).
20. Known history of significant claustrophobia, previous intolerance of CMR imaging or known (or suspected) incompatible metallic implant.
21. Pregnancy.
22. Allergy to gadolinium-based contrast agents used for CMR.
23. Patients with known LVH caused by another established pathology diagnosed prior to or at screening e.g. severe aortic stenosis, hypertrophic cardiomyopathy, amyloidosis and Fabry disease.

Exceptions to the exclusion criteria:

- For criteria 18, patients can enter the trial if they discontinue the use of anti-microbial mouthwash for the duration of the clinical trial.

- Criteria 20 and 22 do not apply to participants who will not have a CMR scan in the NITRATE-CBP arm.

Study design and intervention

This is a prospective double-blind, placebo-controlled, clinical study. A total of 160 patients (male and female, age 18–80) with hypertension as per requirements indicated above will be recruited. Figure 1 shows a summary of the study scheme. Patients will be stratified at screening into 2 arms. Participants with LVH at screening echocardiography will enter the NITRATE-LVH arm (n=80) whilst those who do not have LVH but satisfy the other eligibility criteria will enter the NITRATE-CBP arm (n=80). LVH is defined by increased LV mass determined by acquiring a parasternal long axis image and subsequently measured for the interventricular septal wall thickness, end-diastolic LV cavity diameter and posterior LV wall thickness according to published guidelines.[29] LV mass is calculated as previously described and indexed to body surface area (BSA).[30]

All visits will take place at the William Harvey Heart Centre, Queen Mary University of London and Barts Heart Centre, St Bartholomew's Hospital, Barts Health NHS Trust. Patients will be randomised to receive 70 mL of a beetroot juice concentrate containing ~6 mmol nitrate or nitrate-depleted placebo juice concentrate (James White Drinks, UK) control. The volunteers will start taking their daily dose after completion of their baseline visit (visit 1) for 16 weeks. Patients will be advised to take their dose of juice at the same time each day, preferably in the morning with their breakfast. Patients will be provided with dietary advice in relation to the calorific content of interventions (both ~100kcal). In addition to baseline and 16 week visits, patients will return to the clinic at 8 weeks for collection of samples for NOx analysis and for discussion of any adherence issues.

Randomisation and blinding process

Patients will be block randomised on a 1:1 basis to receive either dietary nitrate or placebo, using a binary random number sequence (http://www.random.org). Treatment assignment for volunteers in the dietary nitrate and placebo groups will remain blinded until data lock and statistical analysis at the end of the study. If unblinding is required, the chief investigator for the study will be informed. A list of the unblinded treatments will be kept in a secure location at the Barts Cardiovascular Clinical Trials Unit (CVCTU). The unblinding procedure will be available at all times.

Patient and public involvement

Patients were directly involved in the design and planning of the study. Twenty previous Barts Health NHS Trust patients were surveyed regarding aspects of trials involving dietary interventions, which informed this protocol.

Study start and end dates

Recruitment commenced on 23rd March 2017. The provisional end date for recruitment is September 2020.

Methods to be used

Blood, saliva and urine analysis

In this study, venous blood will be taken using a 21-gauge butterfly needle at each visit. Blood samples will be centrifuged immediately and plasma separated. The plasma samples will be snap frozen in liquid nitrogen and stored at -80°C. These samples will be used for the purposes of making biochemical measurements and will be discarded once used. Similarly, saliva and urine will be collected at each visit. Saliva will be centrifuged, and a pellet generated. This pellet contains oral bacteria that have dislodged from the oral cavity. This pellet will be frozen for later analysis of oral bacteria by second-generation genome sequencing.

Nitrate and nitrite levels in saliva, blood and urine samples will be determined using ozone chemiluminescence as previously described.[31,32] In brief, total nitrate and nitrite concentration (termed 'NOx') will be determined by adding samples to 0.1mol/L vanadium (III) chloride in 1M hydrochloric acid refluxing at 95°C under nitrogen. Nitrite concentration will be determined by the addition of samples to 0.09 mol/L potassium iodide in glacial acetic acid under nitrogen at room temperature. Nitrate concentration will be calculated by the subtraction of [nitrite] from [NOx]. All measurements will be conducted by an individual blinded to the intervention. In addition, a sample from each batch of juice sent to each volunteer will be analysed for NOx content.

Pulse wave analysis and pulse wave velocity

Pulse wave analysis (PWA) and PWV are measures of arterial stiffness, which will be determined by a non-invasive Vicorder device (Skidmore Medical Limited, Bristol, UK). For PWA, the pulse wave will be recorded from the brachial cuff applied to the non-dominant arm. For PWV, the pulse wave will be simultaneously recorded from the carotid and femoral site using an oscillometric method. A small, inflatable neck pad will be placed directly over a carotid artery and secured around the neck by a Velcro tab and a cuff will also be placed around the patient's ipsilateral upper thigh. Both carotid and

femoral cuffs will be simultaneously inflated automatically to 65 mm Hg and the corresponding oscillometric signal from each cuff digitally analysed to extract the pulse time delay. To estimate the aortic length, the distance between the sternal notch and the thigh cuff will be measured. From these measurements, PWV can be derived as PWV = aortic distance/pulse time delay.[33]

Flow-mediated dilation

FMD will be used to non-invasively assess endothelial function, using vascular ultrasound to measure the increase in brachial artery diameter in response to increased flow[34] as previously described.[35] A high-resolution external vascular ultrasound Siemens/Acuson Sequoia C256 Colour Doppler with a 7.0-MHz linear-array transducer supported by a stereotactic clamp will be used to assess the vessel diameter in the right arm. The vessel will be scanned in longitudinal section and the centre will be identified when the clearest views of the anterior and posterior artery walls have been obtained. Images will be magnified with a resolution box function and images of the brachial artery acquired continuously using semi-automated edge detection software (FMD Studio, Quipu s.r.l, Pisa, Italy) and analysed in real time. An automatic mathematical contour tracking operator locates the pulsed-wave Doppler of the brachial artery which will be used to measure the diameter and blood flow velocity continuously for 1 minute at baseline, then during 5 minutes of reduced blood flow (induced by inflation to 300 mm Hg of a pneumatic cuff placed on the right forearm site distal to the segment of artery being analysed), and finally for a further 5 minutes during reactive hyperaemia following cuff release. FMD is defined as the maximum percentage increase in vessel diameter during reactive hyperaemia. This procedure will be performed at visit 1 (baseline) and visit 3 (16 weeks). In some volunteers, following FMD, 0.4 mg of sublingual GTN (glyceryl trinitrate) will be administered at visit 1 and visit 3 to determine whether changes in FMD responses following intervention might be due to changes in smooth muscle reactivity.

Transthoracic echocardiography

At visit 1 and 3, echocardiography is also performed to assess LV diastolic function. Images will be acquired for measures of LV diastolic function including left atrial end-diastolic volumes, pulmonary vein s/d ratio, mitral valve E/A ratio and E/e'.[29,36] Images will be acquired and analysed by an operator accredited by the British Society of Echocardiography.

CMR

The most accurate and reproducible measurements of LVH are made by CMR.[37] At visits 1 and 3, CMR in the NITRATE-LVH arm and those that consent in the NITRATE-CBP arm will be used to assess

LV mass, volumes and ejection fraction, T1 and T2 mapping, patterns of late gadolinium enhancement (LGE), aortic distensibility and PWV. Participants will have a peripheral venous cannula inserted and gadolinium injected at doses up to 0.2 mmol/kg.

To allow a thorough assessment of cardiac function, the following imaging sequences will be undertaken:

- Scout images, including black and white blood transverse slices of the thorax
- Long axis cines (4-chamber, 2-chamber, LVOT-1, LVOT-2, aortic valve short axis)
- Short axis cine stack
- T1 and T2 mapping
- LGE in the 4-chamber, 2-chamber, 3-chamber views and short axis stack
- Post contrast T1 mapping for extra-cellular volume (ECV) quantification
- Sagittal aortic views and aortic flow sequences for assessment of aortic distensibility and PWV.

Short axis images will be acquired using standard sequences i.e. 10 – 12 slices of 8mm thickness with 2mm gap to achieve whole LV coverage. This is undertaken with the patient in held expiration. Cine and LGE short axis stacks will be acquired using identical scanning geometry to assure correct image registration.

The following offline parameters will be measured:

- Presence of significant extra cardiac pathology
- Left ventricular ejection fraction, volumes and mass
- Distribution of LGE
- Analysis of pre and post contrast T1 mapping and T2 mapping. Short axis images will be segmented according to the AHA model as previously described[38]
- Aortic distensibility and pulse wave velocity.

For the analysis of study outcomes, each study will be anonymised by a third party. This will be separate from the study ID to avoid bias between pre- and post-treatment studies. Documentation of this anonymisation will be stored on a secure server at the Barts CVCTU, with unblinding for clinical reasons as necessary. The PI will be informed if this is necessary. Study analysis will occur in batches to prevent bias. For the assessment of LV volumes and mass, 2 readers will undertake assessment of

inter and intra observer variation prior to analysis of blinded study images. Image analysis will be overseen and adjudicated by an experienced level 3 CMR reader (JCM).

End of study definition

The study will end after the final visit at week 16.

STATISTICAL ANALYSIS

In this study, we intend to recruit 80 patients in each treatment arm with stratification of 80 patients overall recruited to NITRATE-LVH and 80 recruited to the NITRATE-CBP arms (total n=160). This sample size will empower our trial to test for the primary and all major secondary endpoints listed below.

We determined the sample size using G*Power 3.0. Calculations were based on unpaired t-tests, a significance level of 0.05 (two-tailed) and the relevant standard deviations of the mean difference from published studies with reproducibility data: central systolic BP 1.7 mm Hg,[39] LV mass 9.9g,[40] CMR PWV 0.45 m/s,[41] Vicorder PWV 0.29 m/s.[41] For BP, we used measures of SD of systolic BP (as it has greater SD than diastolic BP) from the placebo limb of our previous intervention trial in hypertensive patients[13]: clinic systolic BP 8.4 mm Hg; ambulatory systolic BP 4.9 mm Hg; home systolic BP 3.9 mm Hg. International consensus documents on the optimal method for ultrasound FMD suggest using lab-specific data in sample size calculation and have suggested that 40 participants in a parallel study are sufficient to determine statistically important differences.[35] In a study of 4 week dietary nitrate intervention in hypertensive patients, SD of repeated measures of FMD in the placebo group was 0.6, whilst the mean difference between the groups was 1.0%[13], a SD of 1.1 .

Primary endpoints

For the individual stratified sub-groups:

NITRATE-LVH: For the primary endpoint of LV mass regression we have a power of 95% for detecting a 9g LV mass change (~6% change for a mildly hypertrophied heart of 150 g). Previously published data showed a 10% reduction in LV mass in hypertensives after 3 months treatment with spironolactone.[42] Furthermore, dietary advice aimed at BP reduction in the TOMHS study demonstrated mean 9/9 mm Hg BP reduction and ~20g LV mass reduction from baseline ~200g (by echocardiography) at 3 months post intervention, representing ~10% reduction in LV mass.[43]

NITRATE-CBP: For non-CMR PWV we have a power of > 95% for detecting a 0.6 m/s absolute change (previously demonstrated following 4 weeks dietary nitrate in hypertensive patients[13]). For CMR determined PWV we have a power of > 95% for detecting a 0.6 m/s absolute change.[13] For our primary end point of reduction in central systolic BP we expect a scaled reduction in BP relative to observed decreases in brachial BP (8.7 mm Hg clinic brachial systolic BP)[13] though evidence suggests that low-dose intravenous nitrite infusions act to selectively lower central BP in preference to brachial BP and therefore expected differences may be larger.[44] Nevertheless, using our conservative estimate based on two-thirds brachial BP effect, we have > 90% power to detect a 5.8 mm Hg difference in central systolic BP.

In post hoc analyses data will be combined from the 2 groups to assess the overall impact of inorganic nitrate versus placebo on the entire cohort for BP, nitrate and nitrite analysis. In addition, correlation analyses will be conducted using Pearson's correlation between measures of NOx, LV mass, BP and PWV.

Secondary endpoints

a) Ultrasound FMD (%), > 90% power for detecting a 1.0% absolute change from a reduced FMD of ~5%.[13]

b) Brachial BP

i) Clinic > 85% power to detect 7 mm Hg absolute change.[13]

ii) Ambulatory > 95% power to detect 7 mm Hg absolute change.[13]

iii) Home > 85% power to detect 7 mm Hg absolute change.[13] The change observed in our 4 week study was ~7 mm Hg[13] but with reduced measurement frequency, the possibility of increase measurement variability and therefore SD was arbitrarily doubled for this calculation.

Our sample size calculations assume a drop-out rate of 15% (non-compliance with dietary intervention or follow-up visits) based on our centres previous observations.

Data will be analysed on an intention to treat basis. The data will be compared by multivariable regression analysis for each functional measure. The change from baseline in pre-specified primary, secondary and exploratory outcomes will be compared between groups by Student's t-test. We will adjust the results for multiple variables: sex, age, weight, body mass index and the initial differences between groups in LV mass.

Ethical considerations

The NIHR Barts Biomedical Research Centre Patient and Public Advisory Group reviewed this protocol prior to submission to the Research Ethics Committee (REC). The study protocol and any subsequent amendments, along with materials provided to participants and advertising material, was submitted to London - City and East REC. Written approval from the REC was obtained along with final sponsorship and NHS Health Research Authority (HRA) approval.

Safety considerations

The intervention is 70 mL of beetroot juice concentrate or nitrate-deplete beetroot juice (placebo; James White Drinks, UK). The nitrate is extracted using the same anion exchange technique to remove inorganic nitrate from the general drinking water supplies. There are no known serious side effects from these interventions and nitrate-free juice is classified as a foodstuff. In addition, several recent publications using the placebo juice are now available.[45–47] In the unlikely event of a serious adverse event (SAE) occurring directly as a result of the intervention, this would need to be reviewed by the chief investigator and the procedures followed as described below.

Safety reporting

An AE will be documented in the participants' medical notes and the case report form (CRF) and followed up by the investigators. Serious adverse events (SAEs) will be reported to the sponsor and REC where in the opinion of the chief investigator the event was either 'related' (i.e., resulted from administration of research procedures) and 'unexpected' (i.e., the type of event is not an expected occurrence).

SAEs considered to be 'related' and 'unexpected' will be recorded in the participants' notes, the sponsor SAE form and reported to the sponsor's joint research management office (JRMO) within 24 hours of the research team being notified, and to the main REC within 15 days. The co-investigators will be authorised to sign SAE forms in the absence of the PI. The intervention for the participant will be unblinded in the reporting of an 'unexpected and related' SAE, performed by an individual independent of the study procedures and will allow the investigators to remain blinded. The unblinding of single cases will only be performed if necessary for the safety of a participant.

MONITORING

Trial Steering Committee (TSC)

The TSC is composed of three independent experts in the fields of: cardiac MRI, hypertension and clinical trials along with the investigators and the data monitor and two lay members. This committee met before participant recruitment and will meet annually to assess safety, feasibility or any other arising problems and their recommendations followed.

Data Safety and Monitoring Board (DSMB)

The trial is classified as low-risk and does not require the formal setting up of a DSMB, however a DSMB has been established comprising a Clinical Trials Physician, Statistician and Cardiovascular Physician. The DSMB met to review data after 20 patients in each study arm had completed their involvement, to advise about planned sample size and safety signals. The study will be subject to monitoring by the Sponsor, Queen Mary University of London, in accordance with their policies. Any monitoring findings will be relayed to the TSC by the Chief Investigator and acted in the best interest of patients, sponsor and funder.

Ethics and dissemination

The study will be performed in agreement with the Declaration of Helsinki and is approved by the Local REC (London – City and East; 10/H0703/98). Data collection will be completed by the third quarter of 2020. Primary and secondary analysis will start immediately after data collection is completed, with an aim to prepare publications for submission in 2021. The results of the trial will be published according to the CONSORT statement. Dissemination of results will be in peer-reviewed journal publications and presentations at national and international conferences. NITRATE-TOD is registered on Clinicaltrials.gov (NCT03088514).

Summary

This is the first randomised-controlled trial assessing the use of prolonged dietary nitrate treatment in hypertension-induced target organ damage, specifically LVH and arterial stiffness. This study will determine the potential of prolonged dietary nitrate as adjunctive therapy in patients with sub optimally controlled hypertension on anti-hypertensive medication.

Contributors: All authors listed above fulfil all three International Committee of Medical Journal Editors (ICMJE) guidelines for authorship, which are (1) substantial contributions to conception and design, acquisition of data or analysis, and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. AA raised the funding. AA and VK designed the study. CWZL was responsible for coordinating the

contribution of all authors to this paper. AA, VK, JM and CWZL developed the protocol. CWZL and AJPH are involved the acquisition of data or data analysis. CWZL, KR, JC, AS and CP were responsible for drafting this paper. AM, CD, JM, VK and AA were responsible for editing and providing guidance on the paper. All authors were responsible for critically revising the paper. All authors approved the final version of this paper for submission. This study is supported by the CVCTU, a branch of the Barts CTU UKCRC Reg No. 4.

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Competing interests: AA is a co-director of Heartbeet Ltd, which is a start-up company that seeks to identify commercial potential of dietary nitrate.

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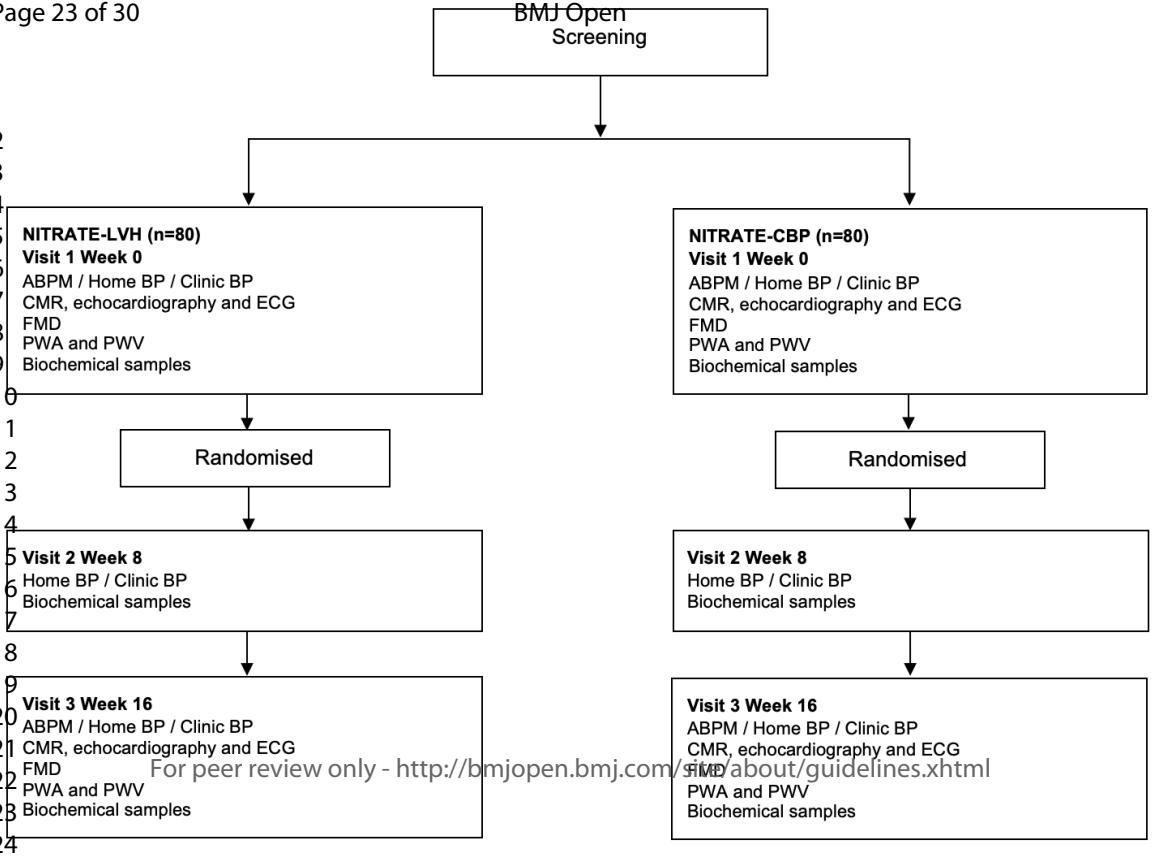
Figure 1 Legend

Figure 1. Study protocol. ABPM, ambulatory blood pressure monitor; ECG, electrocardiogram; CMR, cardiovascular magnetic resonance imaging; FMD, flow mediated dilation; PWA, pulse wave analysis; PWV, pulse wave velocity.



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page	Line no.
Administrative information				
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2	31
	2b	All items from the World Health Organization Trial Registration Data Set	In full protocol	
Protocol version	3	Date and version identifier	In full protocol	
Funding	4	Sources and types of financial, material, and other support	17	9
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1	5
	5b	Name and contact information for the trial sponsor	1	16
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	In full protocol	

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	16	1
Introduction				
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4	78
	6b	Explanation for choice of comparators	5	15
Objectives	7	Specific objectives or hypotheses	6	2
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9	5
Methods: Participants, interventions, and outcomes				
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9	15
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7	2
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9	16
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	15	32

	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9	22
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA	NA
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6	19
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1	
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13	11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6	10
Methods: Assignment of interventions (for controlled trials)				
Allocation:				
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9	25
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9	25
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	In full protocol	

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9	5
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9	25
Methods: Data collection, management, and analysis				
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10	11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14	32
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	In full protocol	
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13	9
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13	9
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13	9
Methods: Monitoring				

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	16	9
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	In full protocol	
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15	20
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	In full protocol	
Ethics and dissemination				
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	15	4
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	In full protocol	
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	In full protocol	
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	In full protocol	

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	17	11
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	In full protocol	
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	In full protocol	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16	18
	31b	Authorship eligibility guidelines and any intended use of professional writers	16	33
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA	
Appendices				
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	In Supplement	
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	10	11

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	9
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	7
Participants	4a	Eligibility criteria for participants	7
	4b	Settings and locations where the data were collected	9
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	13
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	9
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	9
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	9
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	9
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	9

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	9
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	14
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	14
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	NA
	13b	For each group, losses and exclusions after randomisation, together with reasons	NA
Recruitment	14a	Dates defining the periods of recruitment and follow-up	NA
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	NA
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	NA
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	NA
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	NA
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	NA
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	NA
Other information			
Registration	23	Registration number and name of trial registry	16
Protocol	24	Where the full trial protocol can be accessed, if available	16
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	17

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.