Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2026, **17**, 2372

The dose–response effects of nitrate-rich beetroot ingestion on cardiovascular and endothelial function: a randomised controlled trial

Abbie G. McLellan,[a,b] Jarred P. Acton,[a,c] Emma O'Donnell,[a] Samantha N. Rowland,[a] Anthony I. Shepherd,[d] Maria Perissiou,[d] Lewis J. James,[a] Tom Clifford[a] and Stephen J. Bailey*[a]

Dietary nitrate (NO_3^-) supplementation has been reported to improve cardiovascular health, but beyond its effects on brachial artery blood pressure (BP), dose–response effects on other cardiovascular variables are unclear. This study assessed the effects of three acute NO_3^- doses (200 mg, 400 mg, 800 mg NO_3^--rich beetroot powder) on brachial and aortic BP, arterial stiffness and macrovascular endothelial function, in a double-blind, randomised, crossover design. Cardiovascular variables and venous blood samples were measured prior to (control) and 2.5 h post supplement ingestion. Dietary NO_3^- supplementation increased plasma [NO_3^-] and plasma [nitrite] but had no effect on cyclic guanosine monophosphate (cGMP) concentration. Arterial stiffness markers improved following all NO_3^- doses, with no between-dose differences. However, endothelial function only improved following 400 mg (+3.07% compared to control) and aortic systolic BP only improved following 800 mg (–4 mmHg compared to control) dietary NO_3^- supplementation. Acute NO_3^- ingestion improved some cardiovascular risk factors, including arterial stiffness, macrovascular endothelial function and aortic systolic BP with different dose–response effects, but had no effect on brachial BP or plasma [cGMP]. These findings improve our understanding of NO_3^- supplementation and cardiovascular function in healthy adults.

Received 5th December 2025,
Accepted 8th February 2026

DOI: 10.1039/d5fo05298j

rsc.li/food-function

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide, with endothelial dysfunction, arterial stiffness and hypertension established risk factors for CVD morbidity and mortality.[1,2] Nitric oxide (NO) is a multifaceted physiological signalling molecule in the cardiovascular system, with reduced capacity for NO synthesis implicated in the aetiology of endothelial dysfunction, arterial stiffness and hypertension, and therefore, CVD.[3,4] Historically, NO production was attributed, exclusively, to NO synthase (NOS) enzymes, with nitrate (NO_3^-) and nitrite (NO_2^-) considered as biologically inert derivatives of NO oxidation.[5] It has since been demonstrated that NO_3^- and NO_2^- can be recycled back to NO *via* the NO_3^-–NO_2^-–NO pathway.[6] Orally ingested dietary NO_3^- is absorbed almost entirely through enterocytes in the small intestine where it enters systemic circulation.[7] However, the majority is then extracted by the kidneys and excreted in urine (~60%) in first-pass metabolism. Nonetheless, ~25% of ingested NO_3^- is taken up into the salivary glands and secreted into the oral cavity where it is reduced to NO_2^- *via* NO_3^--reducing bacteria. In the stomach, NO_2^- is then reduced to NO and other reactive nitrogen intermediates. As a result, there has been great interest in dietary supplementation with inorganic NO_3^-, which is abundant in beetroot (BR) and green leafy vegetables, as a nutritional NO precursor and cardioprotective nutrient.[8,9] It has been reported that NO_3^- supplementation can lower blood pressure (BP) and arterial stiffness and improve endothelial function in healthy adults[2] and in people with chronic disease,[10] offering broad support for the cardioprotective potential of this nutritional intervention.[11]

Brachial artery BP has been the most frequently evaluated indicator of cardiovascular function following NO_3^- supplementation, though the reported effects have been inconsistent. Indeed, whilst some previous studies have demonstrated 3–9 mmHg reductions in systolic BP (SBP) and 3–6 mmHg reductions in diastolic BP (DBP) following acute ingestion of

[a]*School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, LE11 3TU, UK. E-mail: s.bailey2@lboro.ac.uk*
[b]*University of Oxford Medical Sciences Division, Oxford Cardiovascular Clinical Research Facility, Oxford, OX3 9DU, UK*
[c]*Department of Higher Education Sport, Loughborough College, Loughborough, LE11 3BT, UK*
[d]*School of Psychology, Sport and Health Sciences, University of Portsmouth, Portsmouth, PO1 2UP, UK*

~330–1488 mg NO_3^- in healthy individuals,[12–17] other studies have shown no change in SBP or DBP following acute ingestion of ~400–830 mg NO_3^- in this population group.[18–20] From a dose–response perspective, Wylie *et al.*[14] observed that SBP was reduced after acute ingestion of ~260 mg NO_3^-, decreased to a greater extent with ~520 mg NO_3^-, but showed no further reduction with a higher dose of ~1040 mg NO_3^-, indicating a plateau in the SBP-lowering response.

Compared to brachial BP, the effects of NO_3^- ingestion on aortic BP, arterial stiffness variables, and macrovascular endothelial function are less clear. In healthy participants and in individuals with chronic conditions, aortic SBP has been reduced by up to 6 mmHg following a single dose of ~500–820 mg NO_3^- (ref. 20–22), but another study reported no effect.[23] Similarly, it has been reported that arterial stiffness does not change[20,24] or is reduced (improved) by up to 3.3%[2,25] following acute ingestion of ~830–1533 mg NO_3^-. Additionally, and despite being an established CVD risk factor,[1] relatively few studies have investigated the effect of NO_3^--rich BR supplementation on macrovascular endothelial function.[10,26] Flow mediated dilation (FMD) is an important marker of NO-mediated endothelial function, which has been reported to increase by ~1–3% following acute ingestion of ~310–1400 mg NO_3^- in some studies,[17,27–30] however an acute dose of ~512 mg potassium NO_3^- had no effect on FMD in healthy young adults in another study.[31] Interpreting the results of studies investigating the effect of NO_3^- on cardiovascular function is complicated by inter-study differences in variables which can alter the efficacy of NO_3^- supplementation to improve cardiovascular health variables (BP, arterial stiffness and endothelial function), including NO_3^- dose[12,14,24] and form of supplement,[16,32,33] and the population.[34–36] Previous studies assessing effects on brachial BP have suggested that ingestion of higher NO_3^- doses does not necessarily translate to greater improvements in cardiovascular function.[11,14] Nonetheless, a recent meta-analyses including subgroup comparisons reported an improvement in arterial stiffness exclusively following NO_3^- doses >400 mg.[2] However, the dose–response effect of acute NO_3^- ingestion on macrovascular endothelial function, aortic BP and arterial stiffness has yet to be investigated. This is of importance as the prognostic value of aortic BP, arterial stiffness, and macrovascular endothelial function is increasingly recognised,[1,37] making it relevant to establish effective NO_3^- doses to improve these variables.

Although acute NO_3^- ingestion appears to improve cardiovascular health markers,[2,38] the mechanisms that underlie such effects are not entirely clear. It is generally postulated that beneficial effects of NO_3^- ingestion on the cardiovascular system are NO mediated.[3] Classically, NO signalling in the vascular system was considered to function *via* the NO-cyclic guanosine monophosphate (cGMP)-protein kinase G axis,[8,9] with the more recent demonstration that reactive nitrogen intermediates derived from NO can post translationally modify protein thiol groups *via* S-nitrosylation/S-nitrosation.[39] It has recently been reported that circulating S-nitrosothiols are increased after acute NO_3^- ingestion and correlate with the reduction in brachial BP.[40] On the other hand, the effect of NO_3^- ingestion on plasma cGMP content is conflicting.[12,25,41] Further research is, therefore, required to provide insight into the potential biochemical mechanisms by which NO_3^- ingestion may elicit beneficial effects on cardiovascular function.

We aimed to investigate the dose–response relationship between NO_3^--rich BR (200, 400 and 800 mg NO_3^-) and various markers of cardiovascular health and circulating biomarkers relating to NO_3^- metabolism and NO signalling compared to a control condition. We hypothesized that, following acute NO_3^- ingestion, plasma [NO_3^-], [NO_2^-], and [cGMP] would increase dose-dependently, while brachial and aortic BP, arterial stiffness (augmentation index and augmentation pressure) and macrovascular endothelial function would be improved following ingestion of 400 mg and 800 mg, but not 200 mg, NO_3^-.

Methods

Participants

Seventeen healthy males completed this study (Table 1 and Fig. 1). All experiments were performed in accordance with the Human Tissue Act and UK Research and Innovation (UKRI) Guidelines, and procedures in this study were approved by the Loughborough University Research Ethics Approval Human Participants Sub Committee (ethics code: 14925). Participants provided written informed consent prior to study enrolment. None of the participants were smokers or vapers,[42] nor did they use other dietary supplements or antibacterial mouthwash[43,44] from their familiarisation visit until study completion. Participants were normotensive, had no pre-existing health conditions, and were not taking any medications. Experimental trials occurred at the same time of day (±1 h) and participants refrained from high-intensity exercise and caffeine in the preceding 24 h and 6 h, respectively. Participants arrived at each trial 3 h post-prandial and replicated the same diet in the preceding 24 h. From familiarisation to completion of the study, participants were instructed to avoid overconsuming NO_3^--rich foods (beetroot, radishes,

Table 1 Baseline characteristics of seventeen healthy male participants

	Baseline
Age (years)	23 ± 4
Height (m)	1.78 ± 0.80
Body mass (kg)	76 ± 12
BMI (kg m^{-2})	24 ± 3
Brachial SBP (mmHg)	119 ± 4
Brachial DBP (mmHg)	65 ± 7
HR (bpm)	58 ± 9
FMD (%)	6.71 ± 4.72

Age, height, body mass, body mass index (BMI), brachial systolic blood pressure (SBP), brachial diastolic blood pressure (DBP), heart rate (HR), and flow mediated dilation (FMD) at baseline, collected during the familiarisation visit. Data are presented as group mean ± SD.

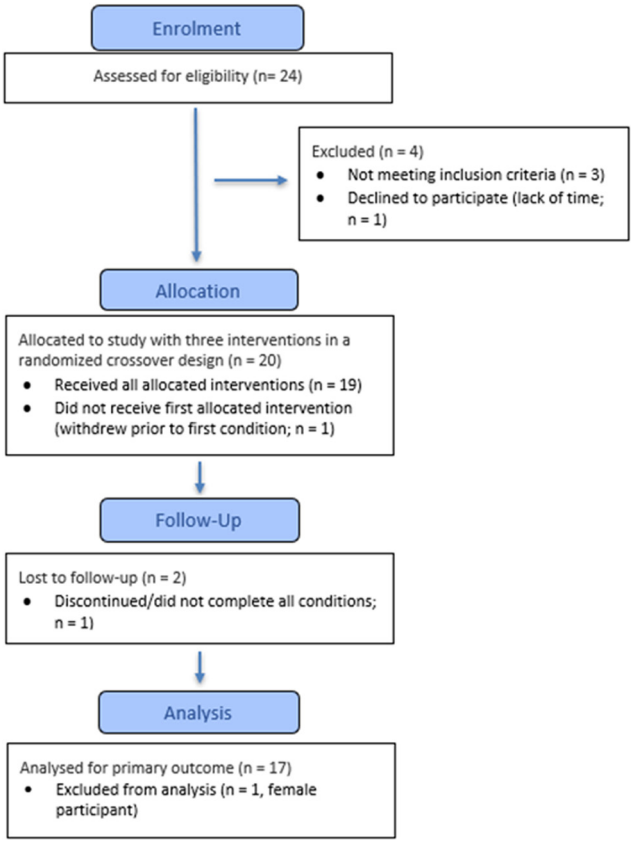

Fig. 1 Flow diagram of participant progress through a crossover randomised controlled trial with three experimental conditions, including information on enrolment, allocation to study, follow-up, and data analysis.

green beans, cress, celery, leafy green vegetables, processed meats) relative to their normal dietary intake of these foods.

Study design

The study adopted a randomised, controlled, double-blind, crossover design, with three supplementation conditions providing 20 g of a formulated BR powder containing either 1% NO_3^- (200 mg), 2% NO_3^- (400 mg) or 4% NO_3^- (800 mg; TruBeet; Bio-gen Extracts Private Limited, Karnataka, India). Experimental visit order was counterbalanced across participants, and supplements were blinded to researchers and participants by someone who was not involved in any of the primary data collection by labelling the supplements in sealed opaque containers with 'A', 'B', or 'C', which were coded to a particular dose.

The 20 g supplement servings contained 13.33 g of formulated BR powder providing 1.5%, 3% and 6% NO_3^- in the 200 mg, 400 mg and 800 mg conditions, respectively. The remaining 6.67 g comprised of a sweetener (stevia), flavour (berry) and flavouring agents (malic & citric acid), included solely to improve palatability and participant compliance. The different BR powders were prepared by manipulating the processing control parameters (extraction time and temperature) during aqueous extraction to ensure precise NO_3^- concentration across the three different conditions, particularly for the low NO_3^-grade material (200 mg). Specifically, raw beetroot was cleaned, shredded and extracted with water for 1 h at >90 °C with the residue obtained from the first extraction processed to obtain the low NO_3^- grade material. The aqueous extract was subsequently concentrated and spray-dried to obtain a free-flowing, water-soluble powder.

The NO_3^- content of BR powders were determined at two stages: first at the beetroot extraction stage and second after blending with flavouring agents and sweetener. NO_3^- quantification was performed on a dried basis using a colorimetric method where samples were treated with a salicylic acid-sulphuric acid mixture, and subsequently 2 N sodium hydroxide, before measuring absorbance using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) at 412 ± 2 nm with values compared to potassium NO_3^- standards.

All BR powders were mixed with 350 mL of water prior to ingestion. Participants attended the laboratory on four occasions, with visit one serving as a familiarisation session and visits two to four serving as the experimental testing sessions. Each visit was separated by at least a 48 h washout.

Familiarisation visit

After initially completing anthropometric measures, participants rested supine for 10 min before two brachial BP measures were completed using an automatic sphygmomanometer (Omron Healthcare, Kyoto, Japan). The FMD procedure was completed with information on the probe and cuff position in relation to anatomical landmarks, image depth, and image diameter recorded for replication in the subsequent experimental visits.

Experimental visits

Each experimental visit comprised a series of measurements assessed both prior to and following supplement ingestion. After 10 min supine rest in a dark, temperature controlled (22.2 ± 1.0 °C) laboratory, brachial BP, aortic BP and arterial stiffness variables, FMD assessments, and a venous blood sample were sequentially collected whilst the participant remained supine. Following this, the participant ingested the BR supplement followed by low NO_3^- water (Buxton mineral water; Buxton, UK) and two cereal bars (Nature Valley; General Mills, US). The participant underwent 2 h and 20 min of seated rest before returning to the laboratory for post-supplement assessments, to coincide with peak plasma [NO_2^-] following acute NO_3^- ingestion.[14] These assessments occurred in the same order as baseline, following a 10 min supine rest in a temperature and light-controlled laboratory.

Measurements

Brachial blood pressure. Brachial BP was assessed using an automatic sphygmomanometer (Omron Healthcare, Kyoto, Japan) on the left arm. BP was recorded 5 times over a 10 min period, while the participant was completely rested. The average of the 5 recordings was calculated and recorded.

Aortic blood pressure and arterial stiffness. An applanation tonometer (SphygmoCor; Atcor Medical, Sydney, Australia) was used to perform pulse wave analysis (PWA) assessed at the left radial artery. Mean brachial SBP and DBP from the prior recordings were used to calibrate the tonometer. A minimum of 2 satisfactory (>80% operator index) PWA recordings were recorded and averaged over 20 consecutive cardiac cycles. Data that failed to meet these criteria were discarded (n = 17 for CON, 200, and 400; n = 16 for 800). PWA variables of interest included: aortic SBP (mmHg), aortic DBP (mmHg), pulse rate (PR, bpm), augmentation pressure (AP, mmHg; the amplitude of the reflected wave), augmentation index (AIx, %; the relative reflected wave amplitude divided by pulse pressure and expressed as a percentage), and AIx adjusted for HR of 75 bpm (AIx@HR75).

Macrovascular endothelial function. Macrovascular endothelial function was assessed *via* FMD (following the assessment and analysis recommendations of Thijssen *et al.*[45] and Corretti *et al.*[46]) in an air-conditioned room (20 °C) in response to a 5 min ischemic stimulus induced by forearm cuff inflation (SC5; Hokanson, Washington, USA). The participant lay supine with their right arm extended horizontally in line with the level of the heart. A manual straight segmental cuff was placed on the forearm, 3 cm distal and inferior to the cubital fossa and the brachial artery was located using a 9 L transducer at 9.0 MHz in an adjustable probe-holding device (LOGIQ E9 Ultrasound; GE Healthcare, Buckinghamshire, UK). Pulse-wave Doppler velocity was acquired simultaneously, with the insonation angle set at 60°. A baseline recording was taken for 1 min, before 5 min manual cuff inflation to 220 mmHg, followed by a final 3 min post occlusive reactive hyperaemia (PORH) recording to capture the peak arterial diameter. Recordings were collected *via* Vascular Imager (Medical Imaging Applications Vascular Research Tools, Iowa, USA) at a rate of 15 frames per second. An experienced sonographer conducted all FMD assessments (1.1% coefficient of variation for diameter, 11.5% coefficient of variation for FMD).

For all FMD analysis, an automated wall detection software package, Brachial Analyser, was used (Medical Imaging Applications Vascular Research Tools, Iowa, USA). The diameter parameters measured were mean brachial arterial diameter at baseline (BAD_{ba}, mm), peak brachial arterial diameter post-occlusion (BAD_{pk}, mm), time to BAD_{pk} (TTP, s), absolute change in brachial arterial diameter (BAD_{FMD}, mm), and relative change in brachial arterial diameter (FMD, %). BAD_{ba} was calculated as the mean value over 30 s. For BAD_{pk}, the three greatest diameters were found, and 3 s averages were calculated. The highest of the three outputs was taken as BAD_{pk}. The time aligned to this point was recorded as the TTP.

The equations for BAD_{FMD} and FMD were:

$$BAD_{FMD}\ (mm) = BAD_{pk} - BAD_{ba}$$

$$FMD\ (\%) = ((BAD_{pk} - BAD_{ba})/BAD_{ba}) \times 100.$$

Shear rate (SR) was calculated, *via* the equation below, at baseline (SR_{base}; reported as the average over 30 s) and PORH (SR_{AUC}; reported as the sum of the area under the curve from cuff release to TTP). FMD (%) was normalised to SR_{AUC} (FMD_{SR}) *via* the following equations:

$$SR\ (s^{-1}) = 4 \times (\text{mean blood flow velocity}/BAD)$$

$$FMD_{SR}\ (\%\ s^{-1}) = FMD/SR$$

Venous blood collection and analysis. Venous blood samples were taken from the antecubital-fossa region, acquiring samples in lithium heparin (LH), ethylenediaminetetraacetic acid (EDTA), and serum vacutainers. All samples were collected (n = 17 for CON, 200 mg and 800 mg) except one post 400 mg NO_3^- (n = 16). Samples were centrifuged at 3500g for 5 min to obtain LH plasma, or 1500g for 15 min to obtain EDTA plasma and serum. Samples were immediately stored at −80 °C for subsequent analysis.

Plasma [NO_2^-] and [NO_3^-] were analysed using LH plasma *via* ozone chemiluminescence. This process is described in full in previous work.[20] In brief, this method of quantification involved deproteinising plasma samples with ethanol prior to subsequent [NO_3^-] analysis. 50 μL deproteinised samples were injected into a purge vessel containing 0.8% (w/v) vanadium chloride in 1 M HCl for determination of plasma [NO_3^-], *via* an ozone-based chemiluminescence NO analyser (Sievers NOA 280i, Analytix Ltd, Durham, UK), by its reduction to NO. For [NO_2^-] determination, plasma samples were deproteinised in ethanol and 200 μL of the supernatant was injected into the purge vessel prepared with glacial acetic acid and aqueous sodium iodide (4% w/v) to quantify plasma [NO_2^-] by its reduction to NO. Plasma [NO_3^-] and [NO_2^-] were determined by plotting signal area (mV) against calibration plots of $NaNO_3$ and $NaNO_2$ standards, respectively. Origin Lab was used to smooth the signal and objectively identify the peaks.

Plasma [cGMP] was measured using EDTA plasma *via* a competitive commercial assay (Cyclic GMP ELISA kit; Cayman Chemical, Michigan, USA).

Statistical analysis

Sample size was estimated with *a priori* power analysis for changes in FMD using G*Power (G*Power 3.1.97; Kiel, Germany). The analysis suggested with a change in means and SD of 1% and 1, respectively, and an assumption of 80% power and a significance level of 0.05, the total number of participants required to detect a statistically significant change in FMD was 17 (large effect size; η_p^2 = 0.20). The mean for the analysis was estimated from similarly designed studies based on a 1% change in FMD being clinically meaningful.[45,47] This is higher than the sample sizes in previous similar studies assessing the effects of a single dose of beetroot juice on cardiovascular function in healthy males that detected statistically significant changes in FMD with 10–11 participants.[27,30] Statistical analysis was performed using Jamovi (version 2.3.28; Jamovi, Sydney, Australia). Visual inspection of Q–Q plots and residual histograms were used to confirm data normality.[48] One-way linear mixed models (LMM) were performed for all variables to account for missing data points. BAD_{ba} was

included as a covariate for LMM analysis of FMD. Four conditions were compared: control (CON, taken as the average of the three experimental visit baseline results), 200 mg NO_3^-, 400 mg NO_3^-, 800 mg NO_3^-. Significant main condition effects were analysed further using Holm–Bonferroni corrected paired *t* tests. To calculate effect sizes, partial eta squared (n_p^2) was used for the omnibus tests, where effects were classified as small ($n_p^2 \geq 0.01$), medium ($n_p^2 \geq 0.06$), or large ($n_p^2 \geq 0.14$).[49] Hedges *g* (*g*) was used for paired-sample's *t* tests, where effects were classified as ($g \geq 0.2$), medium ($g \geq 0.5$), or large ($g \geq 0.8$).[49] All data are displayed as mean ± SD unless stated otherwise. Statistical significance was accepted at $P < 0.05$. All variables for analysis had a sample size of 17.

Results

Plasma [NO_3^-], [NO_2^-], and [cGMP]

There was a main effect of condition for plasma [NO_3^-] ($P < 0.001$, $n_p^2 = 0.973$). *Post-hoc* analyses revealed a significant dose-dependent increase in plasma [NO_3^-] (CON: 50 ± 16 μM, 200: 99 ± 26 μM, 400: 293 ± 42 μM, 800: 482 ± 74 μM; $P < 0.001$, $g = 2.216–7.879$). This represents 2-fold, 6-fold, and 10-fold increases in plasma [NO_3^-] following 200, 400, and 800 conditions, respectively. There was a main effect of condition for plasma [NO_2^-] ($P < 0.001$, $n_p^2 = 0.681$), with plasma [NO_2^-] increasing dose-dependently following 400 mg (300 ± 114 nM) and 800 mg NO_3^- (423 ± 228 nM; $P < 0.008$, $g = 0.666–2.714$). This represents a 4-fold and 6-fold increase in plasma [NO_2^-] following 400 and 800 conditions, respectively. Plasma [NO_2^-] was not significantly different between CON (71 ± 24 nM) and 200 mg NO_3^- (103 ± 48 nM), though the effect size was large ($P = 0.433$, $g = 0.823$). There was no main effect of condition for plasma [cGMP] ($P = 0.087$, $n_p^2 = 0.127$; CON: 4.9 ± 1.5 pmol mL^{-1}, 200: 5.4 ± 2.9 pmol mL^{-1}, 400: 4.3 ± 1.9 pmol mL^{-1}, 800: 4.6 ± 1.4 pmol mL^{-1}; Fig. 2).

Macrovascular endothelial function

Brachial artery diameter. There were no significant differences in BAD_{ba} or BAD_{pk} between conditions ($P = 0.089–0.889$, $n_p^2 = 0.013–0.126$; Table 2). There was a significant main effect of condition for TTP ($P = 0.016$, $n_p^2 = 0.192$; Table 2). *Post-hoc* tests revealed a significant reduction in TTP following 400 ($P = 0.017$, $g = 1.129$), but not 200 ($P = 0.074$, $g = 1.09$) or 800 ($P = 0.430$, $g = 0.376$), compared to CON (Table 2).

Flow mediated dilation. There were significant main effects for condition for BAD_{FMD} ($P = 0.027$, $n_p^2 = 0.172$) and FMD ($P = 0.037$, $n_p^2 = 0.161$). *Post-hoc* tests revealed a greater BAD_{FMD} ($P = 0.036$, $g = 0.671$) and FMD ($P = 0.047$, $g = 0.690$) in 400 mg compared to CON (+0.12 mm and +3.07%; Table 2, Fig. 3). Following 200 mg and 800 mg, BAD_{FMD} ($P = 0.816–0.860$, $g = 0.097–0.395$) and FMD ($P = 1.000$, $g = 0.145–0.335$) were not different from CON (200: +0.01 mm and +0.38%, 800: +0.05 mm and +1.05%; Table 2, Fig. 3). There remained a significant main effect for FMD when accounting for BAD_{ba} as a covariate ($P = 0.025$, $n_p^2 = 0.182$), where FMD was greater in 400 mg ($P = 0.029$, $g = 0.690$), but not 200 mg ($P = 1.000$, $g = 0.145$) or 800 mg ($P = 0.858$, $g = 0.335$) compared to CON. There was a main effect of condition for FMD_{SR} ($P = 0.011$, $n_p^2 = 0.223$). *Post-hoc* analyses revealed greater FMD_{SR} in 400 mg compared to CON ($P = 0.020$, $g = 0.812$) and 200 mg ($P = 0.022$,

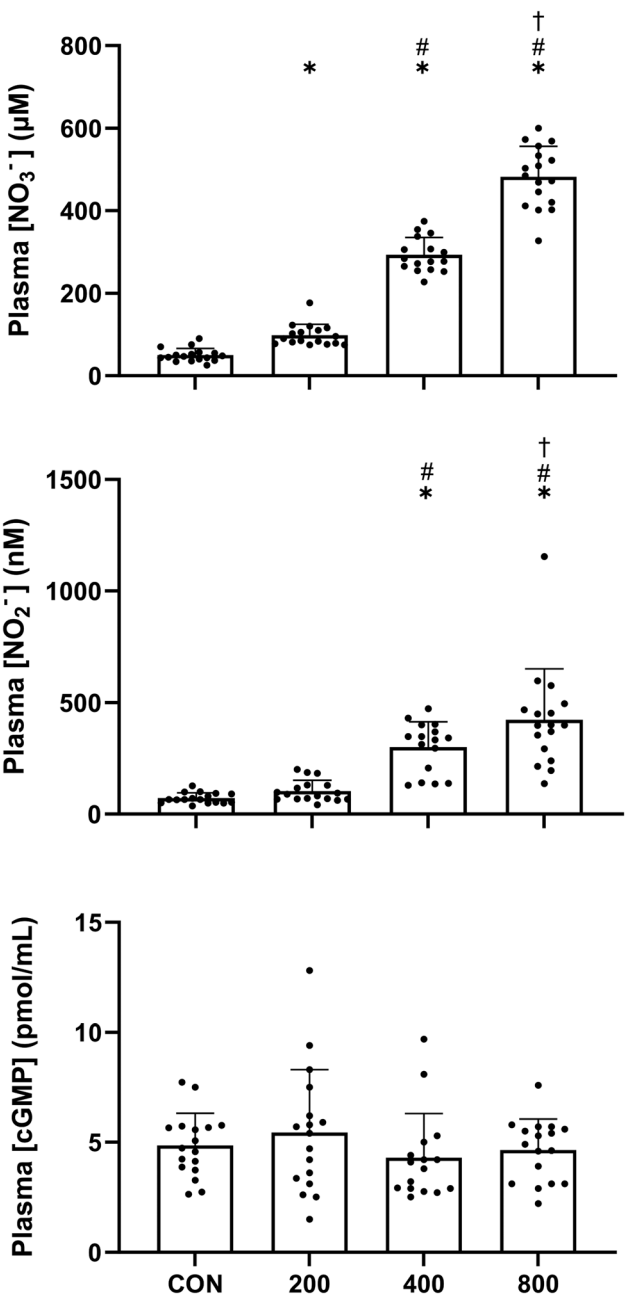

Fig. 2 Plasma nitrate ([NO_3^-]), nitrite ([NO_2^-]), and cyclic guanosine monophosphate ([cGMP]) concentrations following four conditions: no supplement (CON), and NO_3^--rich beetroot powder providing 200 mg (200), 400 mg (400) or 800 mg (800) NO_3^-. The bars represent the group mean ± SD responses with the filled circles representing individual participants. * denotes significantly different from CON ($P < 0.05$). # denotes significantly different from 200 ($P < 0.05$). † denotes significantly different from 400 ($P < 0.05$).

Table 2 Markers of macrovascular endothelial function assessed following four doses of nitrate-rich beetroot juice

	CON	200	400	800
BAD_{ba} (mm)	4.09 ± 0.41	4.13 ± 0.46	4.13 ± 0.46	4.13 ± 0.48
BAD_{pk} (mm)	4.32 ± 0.42	4.37 ± 0.45	4.48 ± 0.50	4.41 ± 0.53
TTP (s)	49 ± 9	40 ± 7	38 ± 10*	44 ± 16
BAD_{FMD} (mm)	0.23 ± 0.09	0.24 ± 0.11	0.35 ± 0.23*	0.28 ± 0.15
FMD (%)	5.61 ± 2.26	5.99 ± 2.84	8.68 ± 5.71*	6.66 ± 3.69
SR_{base} (s^{-1})	19.43 ± 6.83	14.03 ± 4.66	14.76 ± 6.60	16.76 ± 8.86
SR_{AUC} (s^{-1})	2775 ± 838	2589 ± 610	2394 ± 865	2610 ± 906
FMD_{SR} (% s^{-1})	2.15 ± 1.08	2.23 ± 1.05	4.31 ± 3.51*#	2.57 ± 1.64

Brachial artery diameter at baseline (BAD_{ba}) and peak (BAD_{pk}), time to peak diameter (TTP), absolute change in brachial artery diameter (BAD_{FMD}), relative flow mediated dilation (FMD), shear rate at baseline (SR_{base}), accumulative shear rate from post occlusion to TTP (SR_{AUC}), and flow mediated dilation normalised to shear rate (FMD_{SR}) following four conditions: no supplementation (CON; n = 17), and NO_3^--rich beetroot powder providing 200 mg (200; n = 17), 400 mg (400; n = 17) or 800 mg (800; n = 16) NO_3^-. Data are presented as group mean ± SD. * denotes different from CON ($P < 0.05$). # denotes significantly different from 200 ($P < 0.05$).

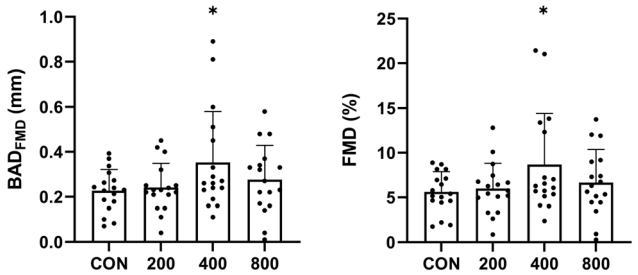

Fig. 3 Absolute change in brachial artery diameter (BAD_{FMD}) and relative flow mediated diameter (FMD) following four conditions: no supplement (CON), and NO_3^--rich beetroot powder providing 200 mg (200), 400 mg (400) or 800 mg (800) NO_3^-. Bars represent the group mean ± SD responses with the filled circles representing individual participants. * denotes different from CON ($P < 0.05$).

Table 3 Aortic blood pressure and arterial stiffness variables following four doses of nitrate-rich beetroot juice

	CON	200	400	800
SBP (mmHg)	99 ± 4	96 ± 7	97 ± 5	95 ± 4*
DBP (mmHg)	65 ± 7	64 ± 9	65 ± 7	63 ± 9
PR (bpm)	57 ± 9	54 ± 9	55 ± 6	53 ± 9
AP (mmHg)	1 ± 3	0 ± 3	−1 ± 4*	−1 ± 4*
AIx (%)	3 ± 10	−1 ± 10	−3 ± 13*	−3 ± 12*
AIx@75HR (%)	−5 ± 8	−11 ± 8*	−12 ± 12*	−13 ± 12*

Aortic systolic blood pressure (SBP), aortic diastolic blood pressure (DBP), augmentation pressure (AP), augmentation index (AIx), and augmentation index relative to heart rate (AIx@75HR) following four conditions: no supplementation (CON), and NO_3^--rich beetroot powder providing 200 mg (200), 400 mg (400) or 800 mg (800) NO_3^-. Data are presented as group mean ± SD. * denotes different from CON ($P < 0.05$).

g = 0.784), but no other between-condition differences (P = 0.081–1.000, g = 0.073–0.620; Table 2).

Aortic blood pressure and arterial stiffness

Aortic blood pressure. There was a significant main effect of condition for aortic SBP (P = 0.025, n_p^2 = 0.178) and *post-hoc* tests revealed a significant reduction in aortic SBP in 800 mg (−4 mmHg; P = 0.032, g = 0.976), but not 200 mg (−3 mmHg; P = 0.119, g = 0.514) or 400 mg (−2 mmHg; P = 0.681, g = 0.431), in comparison to CON (Table 3). There was a main effect of condition for PR (P = 0.043, n_p^2 = 0.157), however *post-hoc* tests revealed no significant between-condition differences (P = 0.068–0.682, g = 0.108–0.434; Table 3). There was no main effect of condition for aortic DBP (P = 0.374, n_p^2 = 0.063; Table 3).

Arterial stiffness variables. There were significant main effects of condition for AIx (P = 0.006, n_p^2 = 0.230), AIx@75HR (P = 0.003, n_p^2 = 0.259) and AP (P = 0.008, n_p^2 = 0.221; Table 3). *Post-hoc* analyses revealed lower AIx and AP in 400 mg (−6% and −2 mmHg; P = 0.010–0.017, g = 0.505–0.552) and 800 mg (−6% and −2 mmHg; P = 0.016–0.019, g = 0.532–0.554), but not 200 mg (−4% and −1 mmHg; P = 0.051–0.114, g = 0.325–0.391), compared to CON (Table 3). AIx@75HR was significantly lower than CON in 200 mg (−6%; P = 0.047, g = 0.732), 400 mg (−7%; P = 0.006, g = 0.670), and 800 mg (−8% P = 0.006, g = 0.770; Table 3). There was no difference in AIx, AIx@75HR, or AP between 200 mg, 400 mg, and 800 mg conditions (P = 0.854–1.000, g = 0.000–0.505; Table 3).

Brachial artery blood pressure

There were no differences in brachial SBP or DBP between conditions (P = 0.151–0.500, n_p^2 = 0.048–0.104; Table 4). There was a significant main effect of condition for HR (P = 0.022, n_p^2 =

Table 4 Brachial blood pressure and heart rate following four doses of nitrate-rich beetroot juice (n = 17)

	CON	200	400	800
SBP (mmHg)	118 ± 5	116 ± 7	117 ± 4	115 ± 5
DBP (mmHg)	65 ± 7	64 ± 9	65 ± 7	63 ± 7
HR (bpm)	58 ± 9	54 ± 9*	56 ± 6	55 ± 9

Brachial systolic blood pressure (SBP), brachial diastolic blood pressure (DBP), and heart rate (HR) following four conditions: no supplementation (CON), and NO_3^--rich beetroot powder providing 200 mg (200), 400 mg (400) or 800 mg (800) NO_3^-. Data are presented as group mean ± SD. * denotes different from CON ($P < 0.05$).

0.180). *Post-hoc* tests revealed that HR was significantly lower in 200 mg (−4 bpm; $P = 0.032$, $g = 0.434$), but not 400 mg (−2 bpm; $P = 0.573$, $g = 0.255$) or 800 mg (−3 bpm; $P = 0.062$, $g = 0.325$), compared to CON (Table 4).

Discussion

The primary original findings of this study were that, compared to CON: (1) macrovascular endothelial function was improved after ingesting 400 mg NO_3^-, but not 200 mg or 800 mg NO_3^-; (2) all NO_3^- doses improved arterial stiffness variables and this improvement was not dose-dependent; (3) aortic SBP was decreased after ingesting 800 mg NO_3^-, but not 200 mg or 400 mg NO_3^-; and (4) plasma [cGMP] was unaffected following ingestion of any of the NO_3^- doses administered. These findings improve understanding of the effects of NO_3^- supplementation on cardiovascular function in healthy, normotensive, young adults by revealing differing dose–response effects on established cardiovascular health markers.

Cardiovascular function

The dose–response relationship between NO_3^- supplementation and BP has been examined previously;[12,14] however, no such investigation had been conducted for NO-mediated macrovascular endothelial function. In the current study, BAD_{FMD} and FMD increased by 0.12 mm and 3%, respectively, after ingesting 400 mg NO_3^- compared to CON. In contrast, neither BAD_{FMD} nor FMD were different between the CON, 200 mg NO_3^- and 800 mg NO_3^- conditions. Consistent with the clinically meaningful increase in FMD following 400 mg NO_3^- in comparison to CON, Yuschen *et al.*[30] and Bakker *et al.*[27] observed 2% increases in FMD following ingestion of 310–400 mg NO_3^- in healthy young adults. Some other studies have reported increased FMD following ingestion of a larger NO_3^- dose (~600–800 mg) in older adults and pregnant females.[28,50–52] It is well established that older age and lower concentration of oestrogen negatively impact macrovascular endothelial function,[51,53] which could account for some of these inter-study differences in the NO_3^- dose required to improve macrovascular endothelial function. Interestingly, Bahra *et al.*[31] observed no change in FMD following ingestion of ~500 mg KNO_3 in young healthy adults, which may be linked to other potential active nutrients in beetroot, such as (poly)phenols, or betalains, that could have contributed to the increase in macrovascular endothelial function observed in the present study.[16,32,54]

The beneficial effects of NO_3^- supplementation on the cardiovascular system have been largely ascribed to the increase in plasma [NO_2^-] and its subsequent potential to either directly nitrosylate/nitrosate protein thiols groups, or to undergo a one-electron reduction to NO.[6] The lack of change in macrovascular endothelial function after ingesting 200 mg NO_3^- in the current study may be a function of an insufficient increase in plasma [NO_2^-]. Indeed, it has been suggested that plasma [NO_2^-] may need to increase above a certain threshold concentration, perhaps 200 nM, before beneficial effects are observed.[55] Consistent with this postulate, plasma [NO_2^-] was 103 ± 48 nM after ingesting 200 mg NO_3^- and macrovascular endothelial function was not improved, whereas macrovascular endothelial function was improved after ingesting 400 mg NO_3^- when plasma [NO_2^-] was 300 ± 114 nM. However, despite a further increase in plasma [NO_2^-] to 423 ± 228 nM after ingesting 800 mg NO_3^-, macrovascular endothelial function was not improved compared to CON. This curtailment in the beneficial effects of NO_3^- dose on endothelial function may be a function of crosstalk between NO derived from endothelial NOS (eNOS) and the NO_3^-–NO_2^-–NO pathway.[56] The FMD technique assesses NO-mediated endothelial function in response to shear stress and the subsequent activation of eNOS.[1,45] However, during the occlusion that precedes the FMD assessment, the decline in brachial artery PO_2 and pH would be expected to aid the reduction of NO_2^- to NO.[57] This potential for increased NO_2^--derived NO after ingestion of 400 mg NO_3^- may have complemented and augmented eNOS-derived NO to increase FMD. However, it is also recognised that elevated NO can interfere with eNOS activity *via* S-nitrosylation.[58] It is possible, therefore, that the higher plasma [NO_2^-] after ingesting 800 mg NO_3^- may have increased NO_2^--derived NO to a greater extent than after ingesting 400 mg NO_3^- which could have impeded eNOS-derived NO such that the net NO generation may have been lower.

Arterial stiffness variables were the most responsive to ingestion of NO_3^--rich BR in the current study, and in contrast to the effects on endothelial function, all NO_3^- doses administered improved AIx@75HR by a similar magnitude, whilst AIx and AP were only improved following 400 mg and 800 mg NO_3^-. Lower AIx has previously been reported following acute ingestion of 400–600 mg NO_3^-, in both low- and high-CVD risk populations[21,36,59] suggesting that NO_3^--rich BR has the potential to lower arterial stiffness. Consistent with a greater potential for acute NO_3^--rich BR supplementation to improve arterial stiffness than endothelial function markers in the current study, a previous study reported improved arterial stiffness but not FMD after acute NO_3^- ingestion in healthy adults.[31] The physiological bases of AIx and AP are not entirely clear,[60] which complicates interpretation of the putative physiological mechanisms that could underpin improved AIx and AP after NO_3^--rich BR supplementation in the current study. Conventionally, AIx and AP have been principally attributed to the timing of reflected waves from the periphery arriving at the aorta, with earlier arrival during systole resulting in higher values, and delayed arrival during diastole resulting in lower values.[61] As such, it is possible that greater potential for NO-induced vasodilation after NO_3^--rich BR supplementation could have mediated the improvements (lowering) in AIx and AP in the current study by increasing arterial compliance, leading to slowing of wave reflections from the periphery, delaying their arrival at the heart.[36] More recently, it has been suggested that AIx and AP are more closely mediated by an

aortic reserve reservoir[62] such that NO_3^--rich BR supplementation may have improved aortic compliance and distensibility through increased NO signalling. An interesting and unexpected observation from the current study was improved AIx@75HR after ingestion of 200 mg NO_3^-. In contrast, previous research[24,34,63] found no improvements in pulse wave variables in healthy adults following the administration of low doses of NO_3^- (70–300 mg). However, since NO_3^- was administered in the form of whole food diets, as opposed to controlled NO_3^--rich BR supplements, and given that the NO_3^- supplementation vehicle can influence NO_3^- metabolism and cardiovascular responses,[16,32,33] this could account for the inter-study differences. Since AIx@75HR was improved with all BR doses administered in the current study, it should also be acknowledged that some other nutrients in the BR powder, such as antioxidants or polyphenols,[64] could have, independently, or synergistically with NO_3^-, contributed to the improvements in arterial stiffness markers in the current study.[16,32,65] Therefore, whilst NO_3^--rich BR supplementation appeared to be particularly potent at improving arterial stiffness markers, the mechanisms that underlie such effects are not entirely clear.

In the current study, aortic SBP was lowered following 800 mg NO_3^-, but 200 mg or 400 mg NO_3^-. This finding is consistent with results from Rowland *et al.*[20] who reported a reduction in aortic SBP in healthy males following acute ingestion of a ~832 mg dietary NO_3^--rich BR dose. However, despite previous reports of a lowering in brachial BP following acute dietary NO_3^- ingestion in healthy participants[12,20,22,29,34,66] no changes in brachial SBP or DBP were observed following any dose of NO_3^--rich BR in the present study. Nonetheless, the findings of the current study are consistent with some previous research.[2,30,50] Since the participants in the current study were normotensive and that the efficacy of NO_3^- supplementation to lower BP appears to diminish with a progressively lower baseline BP,[12,18] this could account for the lack of an effect of NO_3^--rich BR supplementation on brachial BP in the current study.

Dietary nitrate metabolism and NO signalling

In agreement with previous research, plasma [NO_3^-] increased dose-dependently following the acute ingestion of 200 mg, 400 mg, and 800 mg NO_3^--rich BR in the present study.[12,14] However, and in contrast with previous findings,[14] the increase in plasma [NO_2^-] was not dose-dependent, with no significant increase following ingestion of BR providing 200 mg NO_3^-. Nonetheless, plasma [NO_2^-] was increased dose-dependently following ingestion of 400 and 800 mg NO_3^-, consistent with previous observations.[14] Previous research investigating low-dose NO_3^- supplementation *via* the administration of a NO_3^--rich diet, did not investigate plasma [NO_2^-] response,[34] or observed small increases (1-fold) in plasma [NO_2^-].[24] Previous multiple-dose studies have failed to investigate the plasma NO_2^- response in doses as low as the present study.[12,14] Moreover, although there was not a statistically significant increase in plasma [NO_2^-] between 200 mg NO_3^- and CON, the effect size was large, and this was accompanied by improved arterial stiffness. Therefore, a NO_3^- dose as low as 200 mg may be sufficient to improve aspects of cardiovascular function such that the increase in plasma [NO_2^-] elicited by this NO_3^- dose may be clinically relevant. Alternatively, some of the effects of NO_3^- and NO_2^- administration might not be driven entirely by NO_2^-.[15,67] The absence of a change in plasma [cGMP] following any dose of NO_3^--rich BR in the current study implies that vascular improvements were not cGMP-mediated. This agrees with previous work that observed no change in plasma [cGMP] following acute supplementation with 7 mg kg^{-1} potassium NO_3^- in young and older adults with obesity.[41] Conversely, Kapil *et al.*[12] observed significant elevations in plasma [cGMP] 3 h post-supplementation, however, the supplement form and dose administered differed to those in the current study (1488 mg KNO_3), potentially accounting for these disparate effects.

Potential implications

The findings of the present study suggest that acute ingestion of a 200–800 mg NO_3^- dose can improve markers of arterial stiffness whilst a 400 mg dose can improve endothelial function in healthy young adults. Endothelial function is a well-established risk factor for CVD, with an FMD <5% categorised as impaired.[1,45] Meta-analyses have highlighted that a 1% increase in FMD conferred an 8–13% reduction in risk of CVD independent of conventional CVD risk factors.[53,68] Therefore, the 3% mean improvement in FMD in the current study after ingesting 400 mg NO_3^- implies a potentially clinically relevant reduction in CVD morbidity in young healthy males. Moreover, in healthy adults a 1-point standard deviation increase in AIx translates to a 23% rise in risk of cardiovascular mortality[69] and a 10 mmHg reduction in AP translates to a 20% reduction in CVD development.[70] Therefore, the results of the current study imply a potential acute reduction in risk of cardiovascular mortality. Collectively, the current findings suggest that acute ingestion of a BR supplement providing 400 mg NO_3^- can elicit clinically relevant changes in arterial stiffness and endothelial function in support of the potential cardioprotective effects of BR supplementation, at least in healthy young males. Furthermore, since pressures change throughout the arterial tree, brachial BP is not a true reflection of aortic BP and emerging evidence suggests that aortic BP is more predictive of future cardiovascular events than brachial BP.[71] Thus, the current findings provide some evidence to support that acute ingestion of 800 mg NO_3^--rich BR may be cardioprotective.

Experimental considerations

The principal limitation of the current study was that production difficulties with obtaining the low NO_3^- grade BR for a placebo resulted in this study not adopting a placebo-controlled design. Instead, the three NO_3^--rich BR doses were compared to a pre-ingestion CON value. A further potential limitation of this approach is that pre and post BR ingestion assessments were separated by at least 2.5 h, which could have

been confounded by potential circadian effects of the outcome variables. However, this concern is somewhat mitigated by a recent paper suggesting that the efficacy of BR to improve cardiovascular function appears to be consistent across the day with minimal circadian effects on BP and arterial stiffness variables.[20] Whilst the lack of any inter-condition differences in plasma [cGMP] following BR implies that vascular improvements were not cGMP-mediated, since [cGMP] was assessed in venous plasma, it is not possible to exclude the possibility that cGMP may have been elevated in different components of the vascular tree and may have contributed to some of the beneficial cardiovascular effects observed after NO_3^- ingestion in the current study. It has recently been suggested that the increase in whole blood [RSNO] following BR consumption is inversely correlated with the reduction in BP.[15] Therefore, the lack of assessment of blood [RSNO] in the current study is recognised as a limitation.

Conclusions

In young healthy males, acute ingestion of BR providing 400 mg NO_3^- improved macrovascular endothelial function, with arterial stiffness variables comparably improved after ingestion of 200–800 mg NO_3^-. Furthermore, aortic SBP was improved exclusively after ingesting 800 mg NO_3^-. There were no improvements in brachial BP or plasma [cGMP] after ingesting 200–800 mg NO_3^-. These findings improve our understanding of the effect of BR supplementation on risk factors for CVD and suggest that a single BR dose providing 400 mg NO_3^- can elicit clinically relevant improvements in endothelial function and arterial stiffness. While long-term studies are needed to confirm sustained benefits, these data lend further support to the notion that a diet rich in NO_3^--containing vegetables may confer some cardiovascular health benefits.

Author contributions

AGM: conceptualization, formal analysis, investigation, methodology, project administration, resources, visualization, writing – original draft. EOD: methodology, supervision, writing – review & editing. JPA: investigation, writing – review & editing. SNR: methodology, supervision, writing – review & editing. AIS: methodology, validation, writing – review & editing. MP: methodology, validation, writing – review & editing. LJJ: supervision, writing – review & editing. TC: validation, supervision, writing – review & editing. SJB: conceptualization, funding acquisition, methodology, supervision, writing – review & editing.

Conflicts of interest

The authors declare no conflicts of interest regarding this manuscript.

Data availability

The data for this article are available from Loughborough University Research Repository (https://repository.lboro.ac.uk/) at [URL – https://doi.org/10.17028/rd.lboro.30768083].

Acknowledgements

This research was financially supported by a match-funded PhD studentship from Loughborough University and Bio-Gen Extracts (Ltd). Bio-Gen Extracts (Ltd) played no role in the study design, data analysis or interpretation, or the writing of this manuscript. This research was carried out at the National Institute for Health and Care Research (NIHR) Leicester Biomedical Research Centre (BRC). The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

References

1 D. Green, H. Jones, D. Thijssen, N. T. Cable and G. Atkinson, Flow-mediated dilation and cardiovascular event prediction: Does nitric oxide matter?, *Hypertension*, 2011, **57**, 363–369.
2 L. S. Bahrami, S. M. Arabi, Z. Feizy and R. Rezvani, The effect of beetroot inorganic nitrate supplementation on cardiovascular risk factors: A systematic review and meta-regression of randomized controlled trials, *Nitric Oxide*, 2021, **115**, 8–22.
3 K. M. Naseem, The role of nitric oxide in cardiovascular diseases, *Mol. Aspects Med.*, 2005, **26**, 33–65.
4 M. Carlström, J. O. Lundberg and E. Weitzberg, Mechanisms underlying blood pressure reduction by dietary inorganic nitrate, *Acta Physiol.*, 2018, **13080**(224), DOI: 10.1111/APHA.13080.
5 S. Moncada, R. M. J. Palmer and E. A. Higgs, Biosynthesis of nitric oxide from l-arginine: A pathway for the regulation of cell function and communication, *Biochem. Pharmacol.*, 1989, **38**, 1709–1715.
6 J. O. N. Lundberg, E. Weitzberg, J. M. Lundberg and K. Alving, Intragastric nitric oxide production in humans: measurements in expelled air, *Gut*, 1994, **35**, 1543.
7 D. A. Wagner, V. R. Young, S. R. Tannenbaum, D. S. Schultz and W. M. Deen, Mammalian nitrate biochemistry: metabolism and endogenous synthesis, *IARC Sci. Publ.*, 1984, **57**, 247–253.
8 C. P. Bondonno, K. D. Croft and J. M. M. Hodgson, Dietary Nitrate, Nitric Oxide, and Cardiovascular Health, *Crit. Rev. Food Sci. Nutr.*, 2016, **56**, 2036–2052.
9 S. Andrabi, N. Sharma, A. Karan, S. Shahriar, B. Cordon, B. Ma and J. Xie, Nitric Oxide: Physiological Functions, Delivery, and Biomedical Applications, *Adv. Sci.*, 2023, **10**(30), DOI: 10.1002/advs.202303259.

10 A. I. Shepherd, J. T. Costello, S. J. Bailey, N. Bishop, A. J. Wadley, S. Young-Min, M. Gilchrist, H. Mayes, D. White, P. Gorczynski, Z. L. Saynor, H. Massey and C. M. Eglin, 'beet' the cold: Beetroot juice supplementation improves peripheral blood flow, endothelial function, and anti-inflammatory status in individuals with Raynaud's phenomenon, *J. Appl. Physiol.*, 2019, **127**, 1478–1490.

11 V. Kapil, E. Weitzberg, J. O. Lundberg and A. Ahluwalia, Clinical evidence demonstrating the utility of inorganic nitrate in cardiovascular health, *Nitric Oxide*, 2014, **38**, 45–57.

12 V. Kapil, A. B. Milsom, M. Okorie, S. Maleki-Toyserkani, F. Akram, F. Rehman, S. Arghandawi, V. Pearl, N. Benjamin, S. Loukogeorgakis, R. MacAllister, A. J. Hobbs, A. J. Webb and A. Ahluwalia, Inorganic Nitrate Supplementation Lowers Blood Pressure in Humans, *Hypertension*, 2010, **56**, 274–281.

13 A. Vanhatalo, S. J. Bailey, J. R. Blackwell, F. J. DiMenna, T. G. Pavey, D. P. Wilkerson, N. Benjamin, P. G. Winyard and A. M. Jones, Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise, *Am. J. Physiol.: Regul., Integr. Comp. Physiol.*, 2010, **299**, 1121–1131.

14 L. J. Wylie, J. Kelly, S. J. Bailey, J. R. Blackwell, P. F. Skiba, P. G. Winyard, A. E. Jeukendrup, A. Vanhatalo and A. M. Jones, Beetroot juice and exercise: Pharmacodynamic and dose-response relationships, *J. Appl. Physiol.*, 2013, **115**, 325–336.

15 C. Wei, A. Vanhatalo, S. Kadach, Z. Stoyanov, M. Abu-Alghayth, M. I. Black, M. J. Smallwood, R. Rajaram, P. G. Winyard and A. M. Jones, Reduction in blood pressure following acute dietary nitrate ingestion is correlated with increased red blood cell S-nitrosothiol concentrations, *Nitric Oxide*, 2023, **138–139**, 1–9.

16 K. L. Jonvik, J. Nyakayiru, P. J. M. Pinckaers, J. M. G. Senden, L. J. C. van Loon and L. B. Verdijk, Nitrate-rich vegetables increase plasma nitrate and nitrite concentrations and lower blood pressure in healthy adults, *J. Nutr.*, 2016, **146**, 986–993.

17 M. Burleigh, L. Liddle, D. J. Muggeridge, C. Monaghan, N. Sculthorpe, J. Butcher, F. Henriquez and C. Easton, Dietary nitrate supplementation alters the oral microbiome but does not improve the vascular responses to an acute nitrate dose, *Nitric Oxide*, 2019, **89**, 54–63.

18 E. M. Jakubcik, K. Rutherfurd-Markwick, M. Chabert, M. Wong and A. Ali, Pharmacokinetics of Nitrate and Nitrite Following Beetroot Juice Drink Consumption, *Nutrients*, 2021, **13**, 1–14.

19 Z. Jiaqi, D. Zihan, S. Heung-Sang Wong, Z. Chen and E. Tsz-Chun Poon, Acute effects of various doses of nitrate-rich beetroot juice on high-intensity interval exercise responses in women: a randomized, double-blinded, placebo-controlled, crossover trial, *J. Int. Soc. Sports Nutr.*, 2024, **21**, 222–240.

20 S. N. Rowland, L. J. James, E. O'Donnell and S. J. Bailey, Influence of acute dietary nitrate supplementation timing on nitrate metabolism, central and peripheral blood pressure and exercise tolerance in young men, *Eur. J. Appl. Physiol.*, 2023, **124**, 1381–1396.

21 J. A. Chirinos, F. Londono-Hoyos, P. Zamani, M. Beraun, P. Haines, I. Vasim, S. Varakantam, T. S. Phan, T. P. Cappola, K. B. Margulies, R. R. Townsend and P. Segers, Effects of organic and inorganic nitrate on aortic and carotid haemodynamics in heart failure with preserved ejection fraction, *Eur. J. Heart Failure*, 2017, **19**, 1507–1515.

22 D. J. K. Kim, C. A. Roe, Y. B. Somani, D. J. Moore, M. A. Barrett, M. Flanagan, D. B. Kim-Shapiro, S. Basu, M. D. Muller and D. N. Proctor, Effects of acute dietary nitrate supplementation on aortic blood pressures and pulse wave characteristics in post-menopausal women, *Nitric Oxide*, 2019, **85**, 10–16.

23 P. J. Joris and R. P. Mensink, Beetroot juice improves in overweight and slightly obese men postprandial endothelial function after consumption of a mixed meal, *Atherosclerosis*, 2013, **231**, 78–83.

24 D. A. Hobbs, M. G. Goulding, A. Nguyen, T. Malaver, C. F. Walker, T. W. George, L. Methven and J. A. Lovegrove, Acute ingestion of beetroot bread increases endothelium-independent vasodilation and lowers diastolic blood pressure in healthy men: a randomized controlled trial, *J. Nutr.*, 2013, **143**, 1399–1405.

25 S. Velmurugan, J. M. Gan, K. S. Rathod, R. S. Khambata, S. M. Ghosh, A. Hartley, S. Van Eijl, V. Sagi-Kiss, T. A. Chowdhury, M. Curtis, G. G. C. Kuhnle, W. G. Wade and A. Ahluwalia, Dietary nitrate improves vascular function in patients with hypercholesterolemia: A randomized, double-blind, placebo-controlled study, *Am. J. Clin. Nutr.*, 2016, **103**, 25–38.

26 C. M. Eglin, J. T. Costello, S. J. Bailey, M. Gilchrist, H. Massey and A. I. Shepherd, Effects of dietary nitrate supplementation on the response to extremity cooling and endothelial function in individuals with cold sensitivity. A double blind, placebo controlled, crossover, randomised control trial, *Nitric Oxide*, 2017, **70**, 76–85.

27 E. Bakker, H. Engan, A. Patrician, E. Schagatay, T. Karlsen, U. Wisløff and S. E. Gaustad, Acute dietary nitrate supplementation improves arterial endothelial function at high altitude: A double-blinded randomized controlled cross over study, *Nitric Oxide*, 2015, **50**, 58–64.

28 G. V. de Oliveira, M. Morgado, A. P. Pierucci and T. S. Alvares, A single dose of a beetroot-based nutritional gel improves endothelial function in the elderly with cardiovascular risk factors, *J. Funct. Foods*, 2016, **26**, 301–308.

29 A. J. Webb, N. Patel, S. Loukogeorgakis, M. Okorie, Z. Aboud, S. Misra, R. Rashid, P. Miall, J. Deanfield, N. Benjamin, R. MacAllister, A. J. Hobbs and A. Ahluwalia, Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite, *Hypertension*, 2008, **51**, 784–790.

30 X. Yuschen, J. H. Choi, J. Seo, Y. Sun, E. Lee, S. W. Kim and H. Y. Park, Effects of Acute Beetroot Juice Supplementation

and Exercise on Cardiovascular Function in Healthy Men in Preliminary Study: A Randomized, Double-Blinded, Placebo-Controlled, and Crossover Trial, *Healthcare*, 2024, **12**(13), DOI: 10.3390/healthcare12131240.

31 M. Bahra, V. Kapil, V. Pearl, S. Ghosh and A. Ahluwalia, Inorganic nitrate ingestion improves vascular compliance but does not alter flow-mediated dilatation in healthy volunteers, *Nitric Oxide*, 2012, **26**, 197–202.

32 S. T. J. McDonagh, L. J. Wylie, J. M. A. Webster, A. Vanhatalo and A. M. Jones, Influence of dietary nitrate food forms on nitrate metabolism and blood pressure in healthy normotensive adults, *Nitric Oxide*, 2018, **72**, 66–74.

33 E. F. Bowles, M. Burleigh, A. Mira, S. G. J. Van Breda, E. Weitzberg and B. T. Rosier, Nitrate: "the source makes the poison", *Crit. Rev. Food Sci. Nutr.*, 2024, **30**, 1–27.

34 A. H. Liu, C. P. Bondonno, K. D. Croft, I. B. Puddey, R. J. Woodman, L. Rich, N. C. Ward, J. A. Vita and J. M. Hodgson, Effects of a nitrate-rich meal on arterial stiffness and blood pressure in healthy volunteers, *Nitric Oxide*, 2013, **35**, 123–130.

35 V. Kapil, R. S. Khambata, A. Robertson, M. J. Caulfield and A. Ahluwalia, Dietary nitrate provides sustained blood pressure lowering in hypertensive patients: A randomized, phase 2, double-blind, placebo-controlled study, *Hypertension*, 2015, **65**, 320–327.

36 W. E. Hughes, K. Ueda, D. P. Treichler and D. P. Casey, Effects of acute dietary nitrate supplementation on aortic blood pressure and aortic augmentation index in young and older adults, *Nitric Oxide*, 2016, **59**, 21–27.

37 A. Kwon, G.-H. Kim and M.-S. Kim, Clinical implications of central blood pressure measured by radial tonometry and automated office blood pressure measured using automatic devices in cardiovascular diseases, *Front. Cardiovasc. Med.*, 2022, **11**(9), DOI: 10.3389/fcvm.2022.906021.

38 L. C. Gee and A. Ahluwalia, Dietary Nitrate Lowers Blood Pressure: Epidemiological, Pre-clinical Experimental and Clinical Trial Evidence, *Curr. Hypertens. Rep.*, 2026, **18**(17), DOI: 10.1007/s11906-015-0623-4.

39 P. H. MacArthur, S. Shiva and M. T. Gladwin, Measurement of circulating nitrite and S-nitrosothiols by reductive chemiluminescence, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2007, **851**, 93–105.

40 M. Abu-Alghayth, A. Vanhatalo, L. J. Wylie, S. T. McDonagh, C. Thompson, S. Kadach, P. Kerr, M. J. Smallwood, A. M. Jones and P. G. Winyard, S-nitrosothiols, and other products of nitrate metabolism, are increased in multiple human blood compartments following ingestion of beetroot juice, *Redox Biol.*, 2021, **43**, DOI: 10.1016/j.redox.2021.101974.

41 A. W. Ashor, S. Chowdhury, C. Oggioni, O. Qadir, K. Brandt, A. Ishaq, J. C. Mathers, G. Saretzki and M. Siervo, Inorganic nitrate supplementation in young and old obese adults does not affect acute glucose and insulin responses but lowers oxidative stress, *J. Nutr.*, 2016, **146**, 2224–2232.

42 S. J. Bailey, J. R. Blackwell, L. J. Wylie, T. Holland, P. G. Winyard and A. M. Jones, Improvement in blood pressure after short-term inorganic nitrate supplementation is attenuated in cigarette smokers compared to non-smoking controls, *Nitric Oxide*, 2016, **61**, 29–37.

43 M. Govoni, E. Å. Jansson, E. Weitzberg and J. O. Lundberg, The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash, *Nitric Oxide*, 2008, **19**, 333–337.

44 R. Bescos, A. Ashworth, C. Cutler, Z. L. Brookes, L. Belfield, A. Rodiles, P. Casas-Agustench, G. Farnham, L. Liddle, M. Burleigh, D. White, C. Easton and M. Hickson, Effects of Chlorhexidine mouthwash on the oral microbiome, *Sci. Rep.*, 2020, **10**(1), DOI: 10.1038/S41598-020-61912-4.

45 D. H. J. Thijssen, M. A. Black, K. E. Pyke, J. Padilla, G. Atkinson, R. A. Harris, B. Parker, M. E. Widlansky, M. E. Tschakovsky and D. J. Green, Assessment of flow-mediated dilation in humans: a methodological and physiological guideline, *Am. J. Physiol.: Heart Circ. Physiol.*, 2011, **300**, 2–12.

46 M. C. Corretti, T. J. Anderson, E. J. Benjamin, D. Celermajer, F. Charbonneau, M. A. Creager, J. Deanfield, H. Drexler, M. Gerhard-Herman, D. Herrington, P. Vallance, J. Vita and R. Vogel, Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the International Brachial Artery Reactivity Task Force, *J. Am. Coll. Cardiol.*, 2002, **39**, 257–265.

47 A. Cheok, Y. Xu, Z. Zhang, P. W. Caton and A. Rodriguez-Mateos, Betalain-rich dragon fruit (pitaya) consumption improves vascular function in men and women: a double-blind, randomized controlled crossover trial, *Am. J. Clin. Nutr.*, 2022, **115**, 1418–1431.

48 H. Aguinis, R. K. Gottfredson and H. Joo, Best-Practice Recommendations for Defining, Identifying, and Handling Outliers, *Organ. Res. Methods*, 2013, **16**, 270–301.

49 D. Lakens, Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs, *Front. Psychol.*, 2013, **4**(863), DOI: 10.3389/fpsyg.2013.00863.

50 M. A. Walker, T. G. Bailey, L. McIlvenna, J. D. Allen, D. J. Green and C. D. Askew, Acute dietary nitrate supplementation improves flow mediated dilatation of the superficial femoral artery in healthy older males, *Nutrients*, 2019, **11**(5), DOI: 10.3390/NU11050954.

51 M. Volino-Souza, G. V. De Oliveira and T. S. Alvares, A single dose of beetroot juice improves endothelial function but not tissue oxygenation in pregnant women: a randomised clinical trial, *Br. J. Nutr.*, 2018, **120**, 1006–1013.

52 M. Volino-Souza, G. Vieira De Oliveira, V. Dos, S. Pinheiro, C. A. Conte-Junior, T. Da, S. Alvares, A. Conte-Junior and T. Da Silveira Alvares, The effect of dietary nitrate on macro- and microvascular function: A systematic review, *Crit. Rev. Food Sci. Nutr.*, 2022, **64**, 1225–1236.

53 D. H. J. Thijssen, R. M. Bruno, A. C. C. M. Van Mil, S. M. Holder, F. Faita, A. Greyling, P. L. Zock, S. Taddei, J. E. Deanfield, T. Luscher, D. J. Green and L. Ghiadoni, Expert consensus and evidence-based recommendations

for the assessment of flow-mediated dilation in humans, *Eur. Heart J.*, 2019, **40**, 2534–2547.

54 D. A. Hobbs, N. Kaffa, T. W. George, L. Methven and J. A. Lovegrove, Blood pressure-lowering effects of beetroot juice and novel beetroot-enriched bread products in normotensive male subjects, *Br. J. Nutr.*, 2012, **108**, 2066–2074.

55 P. E. James, G. R. Willis, J. D. Allen, P. G. Winyard and A. M. Jones, Nitrate pharmacokinetics: Taking note of the difference, *Nitric Oxide*, 2015, **48**, 44–50.

56 M. Carlström, M. Liu, T. Yang, C. Zollbrecht, L. Huang, M. Peleli, S. Borniquel, H. Kishikawa, M. Hezel, A. E. G. Persson, E. Weitzberg and J. O. Lundberg, Cross-talk between Nitrate-Nitrite-NO and NO Synthase Pathways in Control of Vascular NO Homeostasis, *Antioxid. Redox Signal.*, 2015, **23**, 295–306.

57 J. L. Zweier, H. Li, A. Samouilov and X. Liu, Mechanisms of nitrite reduction to nitric oxide in the heart and vessel wall, *Nitric Oxide*, 2010, **22**, 83–90.

58 K. Ravi, L. A. Brennan, S. Levic, P. A. Ross and S. M. Black, S-nitrosylation of endothelial nitric oxide synthase is associated with monomerization and decreased enzyme activity, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 2619.

59 E. J. Pekas, T. S. K. Wooden, S. K. Yadav and S. Y. Park, Body mass-normalized moderate dose of dietary nitrate intake improves endothelial function and walking capacity in patients with peripheral artery disease, *Am. J. Physiol.: Regul., Integr. Comp. Physiol.*, 2021, **321**, 162–173.

60 M. Shimizu and K. Kario, Role of the augmentation index in hypertension, *Ther. Adv. Cardiovasc. Dis.*, 2008, **2**, 25–35.

61 M. R. Nelson, J. Stepanek, M. Cevette, M. Covalciuc, R. T. Hurst and A. J. Tajik, Noninvasive measurement of central vascular pressures with arterial tonometry: Clinical revival of the pulse pressure waveform?, *Mayo Clin. Proc.*, 2010, **85**, 460–472.

62 M. G. Schultz, A. D. Hughes, J. E. Davies and J. E. Sharman, Associations and clinical relevance of aortic-brachial artery stiffness mismatch, aortic reservoir function, and central pressure augmentation, *Am. J. Physiol.: Heart Circ. Physiol.*, 2015, **309**, 1225–1233.

63 C. P. Bondonno, A. H. Liu, K. D. Croft, N. C. Ward, X. Yang, M. J. Considine, I. B. Puddey, R. J. Woodman and J. M. Hodgson, Short-term effects of nitrate-rich green leafy vegetables on blood pressure and arterial stiffness in individuals with high-normal blood pressure, *Free Radicals Biol. Med.*, 2014, **77**, 353–362.

64 P. C. Wootton-Beard and L. Ryan, A beetroot juice shot is a significant and convenient source of bioaccessible antioxidants, *Proc. Nutr. Soc.*, 2011, **70**(OCE4), DOI: 10.1017/s0029665111001868.

65 W. S. Zoughaib, M. J. Fry, A. Singhal and A. R. Coggan, Beetroot juice supplementation and exercise performance: is there more to the story than just nitrate?, *Front. Nutr.*, 2024, **11**, DOI: 10.3389/FNUT.2024.1347242.

66 L. T. Coles and P. M. Clifton, Effect of beetroot juice on lowering blood pressure in free-living, disease-free adults: a randomized, placebo-controlled trial, *Nutr. J.*, 2012, **11**(1), DOI: 10.1186/1475-2891-11-106.

67 M. Feelisch, T. Akaike, K. Griffiths, T. Ida, O. Prysyazhna, J. J. Goodwin, N. D. Gollop, B. O. Fernandez, M. Minnion, M. M. Cortese-Krott, A. Borgognone, R. M. Hayes, P. Eaton, M. P. Frenneaux and M. Madhani, Long-lasting blood pressure lowering effects of nitrite are NO-independent and mediated by hydrogen peroxide, persulfides, and oxidation of protein kinase G1a redox signalling, *Cardiovasc. Res.*, 2020, **116**, 51–62.

68 Y. Matsuzawa, T.-G. Kwon, R. J. Lennon, L. O. Lerman and A. Lerman, Prognostic Value of Flow-Mediated Vasodilation in Brachial Artery and Fingertip Artery for Cardiovascular Events: A Systematic Review and Meta-Analysis, *J. Am. Heart Assoc.*, 2015, **4**, 1–15.

69 J. H. Janner, N. Skavlan Godtfredsen, S. Ladelund, J. Vestbo and E. Prescott, High aortic augmentation index predicts mortality and cardiovascular events in men from a general population, but not in women, *Eur. J. Prev. Cardiol.*, 2012, **20**, 1005–1012.

70 J. A. Chirinos, J. P. Zambrano, S. Chakko, A. Veerani, A. Schob, H. J. Willens, G. Perez and A. J. Mendez, Aortic pressure augmentation predicts adverse cardiovascular events in patients with established coronary artery disease, *Hypertension*, 2005, **45**, 980–985.

71 C. M. McEniery, J. R. Cockcroft, M. J. Roman, S. S. Franklin and I. B. Wilkinson, Central blood pressure: Current evidence and clinical importance, *Eur. Heart J.*, 2014, **35**(26), DOI: 10.1093/eurheartj/eht565.