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STUDIES IN HUMANS

Dietary nitrate does not modify blood pressure and cardiac output at rest and during exercise in older adults: a randomised cross-over study

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ABSTRACT

Dietary nitrate (NO_3^-) supplementation has been associated with improved vascular and metabolic health. We conducted a double-blind, cross-over, placebo-controlled RCT to investigate the effects of 7-d consumption of beetroot juice compared with placebo on (1) blood pressure (BP) measured in resting conditions and during exercise, (2) cardiac and peripheral vascular function and (3) biomarkers of inflammation, oxidative stress and endothelial integrity. Twenty non-smoking healthy participants aged 60–75 years and BMI 20.0–29.9 kg/m^2 were recruited. Measurement was conducted before and after each 7-d intervention period. Consumption of NO_3^- had no effect on resting systolic and diastolic BP. NO_3^- consumption did not improve indexes of central and peripheral cardiac function responses during cardiopulmonary exercise testing. Dietary NO_3^- supplementation did not modify biomarkers of inflammation, oxidative stress and endothelial integrity. This study does not support the short-term benefits of dietary NO_3^- supplementation on physiological and biochemical markers of vascular health in older healthy adults.

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Nitric oxide; exercise; vascular health; cardiac function; ageing

Introduction

Ageing is a primary risk factor for atherosclerosis and cardiovascular diseases (North & Sinclair 2012). Cardiac ageing is characterised by prominent changes in cardiovascular tissues including hypertrophy, altered left ventricular (LV) diastolic function and cardiac output (CO), and increased arterial stiffness. In older adults, resting CO is preserved by an increase in LV end-diastolic volume with a consequent augmentation of stroke volume (SV). With the increase in energy demands during exercise, older adults achieve people a higher SV and mean arterial blood pressure (BP) but lower heart rate (HR) and peak oxygen consumption compared to younger subjects. Therefore, SV during exercise in older adults is preserved by an increase in end-diastolic volume, whereas in younger subjects is maintained by a progressive decrease in end-systolic volume (Cheitlin 2003; Houghton et al. 2016). Augmentation index (AIx) was significantly higher in older than younger participants and was inversely related to CO in older participants (Houghton et al. 2016).

Nitric oxide (NO) appears to have pleiotropic effects on cardiac physiology (North & Sinclair 2012), being produced by all myocardial cells and is involved in the regulation of coronary vasodilation and cardiomyocyte contractility (Massion et al. 2003). NO is synthesised by vascular and endocardial endothelial nitric oxide synthases (NOS), as well as neuronal and inducible NOS (Rastaldo et al. 2007). The effects of NO on myocardial contractility appear to be mediated by the opening of sarcolemmal voltage-operated and sarcoplasmic ryanodine receptor Ca(2+) channels (Rastaldo et al. 2007). NO is also involved in the modulating post-ischaemic cardiac remodelling infarction which may be mediated by a decreased mitochondrial permeability (Di Lisa et al. 2001).

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Supplemental data for this article can be accessed here.

NO is involved in several other physiological functions such as maintenance of vascular tone, platelet adhesion, angiogenesis, mitochondrial oxygen consumption, muscular performance and control of immunity and inflammation signalling pathways (Kelm 1999). Inorganic NO_3^- represents the final, stable end product of NO metabolism and it is mainly excreted in urine (~70%). Approximately 25–30% of circulating blood NO_3^- enters a non-enzymatic NO synthetic pathway involving salivary glands, oral microbiota and gastric acidic environment (Lundberg et al. 2009). Inorganic NO_3^- from food can also enter the non-enzymatic NO pathway, increase NO production and induce positive effects on cardiovascular function and muscle performance (Lundberg et al. 2009). The role of ageing as a modifier of the effects of inorganic NO_3^- on cardiovascular outcomes remains unknown. Convincing evidence on health benefits of dietary NO_3^- on cardiovascular outcomes currently exists for young- and middle-aged individuals (Lara, Ashor, et al. 2015; Gee & Ahluwalia 2016), whereas contrasting findings have been reported in older populations (Siervo et al. 2013; Lara, Ogbonmwan, et al. 2015; Gee & Ahluwalia 2016; Omar et al. 2016). In addition, limited information is available on the effects of inorganic NO_3^- consumption on cardiac function at rest and during exercise in healthy adults (DeVan et al. 2015; Lee et al. 2015) and in patients with heart disease (Zamani et al. 2015; Eggebeen et al. 2016).

We hypothesise that dietary NO_3^- consumption can increase systemic NO bio-availability and have a positive effect on central and peripheral haemodynamic responses of healthy older adults measured at rest, during different exercise intensities (low, moderate and high) and post-exercise recovery. We evaluated the effects of beetroot juice, chosen as a rich source of dietary NO_3^-, on BP, AIx and haemodynamic parameters of cardiac function including CO, SV, cardiac index (CI) and HR measured at rest and during graded exercise on a stationary bike. We also evaluated whether dietary NO_3^- consumption induced changes in circulating biomarkers of inflammation, oxidative stress and endothelial integrity to provide mechanistic insights into the effects of dietary NO_3^- on circulatory biomarkers closely involved in the regulation of vascular function.

Methods

The trial was approved by the North of Scotland Research Ethics committee (14/NS/0061) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The study was a double-blind, cross-over, placebo-controlled RCT which took place between May and August 2014 across two sites (Newcastle upon Tyne and Sheffield). The duration of the each intervention was 1 week with a wash-out period between treatments of at least 1 week. This trial was registered in the International Standard Randomised Controlled Trial Number Register (ISRCTN19064955).

Participants

Twenty older healthy people (10 males, 10 females) were recruited (10 participants per site). Participants were included in the study if they did not have medical conditions or were not taking medications that might influence the study outcomes. A full list of the inclusion and exclusion criteria is provided in the Online Supplementary Material. Participants were asked to maintain their habitual diet and to avoid using chewing gum or mouth wash for at least 48 h prior to the baseline visits (first and third visit) and during each of the 1-week supplementation periods.

Study overview

A telephone screening was performed to check eligibility to the trial's inclusion and exclusion criteria. Eligible participants were asked to arrive at the research facilities after a 12-h overnight fast and having avoided strenuous physical activity for 3 d preceding the visit. Eligibility to the study was confirmed by measuring BMI, resting BP and conducting a resting 12 lead electrocardiogram. Participants were randomised to a cross-over intervention and the assessment continued with the measurement of body composition and collection of blood and urine samples and the assessment of physical capability (reported elsewhere). Participants then rested for 1 h and consumed a meal providing approximately 300 kcal (CHO = 85%, PRO = 3%, FAT = 12%). After the 1-hur rest period, the exercise test was explained to the participants and they became accustomised to the bicycle ergometer. The exercise protocol is described in Figure S1 of the Online Supplementary Material. After the vascular measurements and the exercise test, instructions were provided for self-administration of the nutritional intervention (14 bottles of either NO_3^- -rich or NO_3^- -depleted beetroot juice; 70 ml ×2/d; Beet It, James White Ltd, Ashbocking, Suffolk, UK) and asked to consume one bottle of beetroot juice each morning

and evening for the subsequent 7 d. The daily dose of NO_3^--rich (intervention) or NO_3^- -depleted (placebo) beetroot juice contained ~12 mmol and ~0.003 mmol of NO_3^-, respectively. This concluded Visit 1 of the trial. Participants returned to the research facilities in the morning of day 8 after they had completed a 7-d supplementation period. Measurements were conducted approximately after 12 h from drinking the beetroot juice as participants were asked to fast overnight before arriving to the research centre. The resting 12 lead ECG was performed and if normal the visit continued with a repeat of the assessments performed at visit 1. At the end of the second visit, participants were asked to resume their habitual diet and physical activity. After a wash-out period of at least 7 d, the second phase (including Visits 3 and 4) was conducted similar to the first phase with the exception that participants crossed-over experimental arms, that is consumed the other intervention agent.

Resting and daily blood pressure

Resting BP was measured in triplicate using an automated BP monitor (Omron M3, Omron Healthcare, Milton Keynes, UK) at each clinic visit with the participant seated comfortably for 15 min prior to the measurement and the arm supported at the level of the heart. The same BP monitor was provided to each participant for the measurements of daily resting BP at home. Participants were asked to conduct duplicate measurements in a seated position in the morning before drinking the juice and in the evening before going to bed. Agreement of the daily BP monitoring was verified against the BP recordings obtained from the 24-h Ambulatory Blood Pressure Monitoring (ABPM) (systolic BP, $r = 0.71$, $p < .001$, $n = 84$; diastolic BP, $r = 0.80$, $p < .001$, $n = 84$) (Jajja et al. 2014).

Resting and exercise central haemodynamics

All subjects performed a maximal graded cardiopulmonary exercise test using an electro-magnetically controlled bicycle ergometer (Corival, Lode, Groningen, The Netherlands) with online gas exchange measurements (Metalyzer 3B, Cortex, Leipzig, Germany). The maximal progressive exercise test included cycling with 10-W increments every minute until volitional exhaustion. The 12-lead ECG (Custo, CustoMed GmbH, Ottobrunn, Germany) was continuously monitored and BP (Tango, SunTech Medical, Morrisville, NC) recorded at rest, during exercise and recovery (Newcastle Centre only, $N = 10$). The test was terminated when the subject was unable to pedal at a cadence of 50 rpm or they reached maximal oxygen consumption. Peak oxygen consumption was defined as the average oxygen uptake during the last minute of exercise. Non-invasive central haemodynamics parameters (SV, CO and CI) were measured by bio-reactance method (NICOM, Cheetah Medical, Newton Center, MA) (Jakovljevic et al. 2014). CO was estimated under resting and exercise stress testing conditions using the bio-reactance method which analysis the frequency of relative phase shifts of electrical current applied across the thorax using four dual-surface electrodes. Signals were applied to and recorded from the left and right sides of the thorax; these signals are processed separately and averaged after digital processing. The signal processing unit of the system determines the relative phase shift between the input signal relative to the output signal. The phase shift occurs due to instantaneous changes in blood flow in the aorta. CO is subsequently estimated as the product of SV and HR. CI is calculated by adjusting the CO for body surface area. A graphical description of the protocol is described in Figure S2 of the Online Supplementary Material.

Augmentation index

A high fidelity micro-manometer was used to apply a gentle pressure and therefore flatten the radial artery in the non-dominant hand at the wrist under resting condition using the SphygmoCor (AtCor Medical, West Ryde, NSW, Australia). Central aortic pressure and AIx were then calculated automatically using the SphygmoCor software. AIx was calculated as the difference between the first systolic peak and the second systolic peak of the central arterial waveform, which was expressed as a percentage of pulse pressure.

Anthropometry, dietary and lifestyle questionnaires

Body weight and height were measured to the nearest 0.1 kg and 0.5 cm, respectively. The nine-item short form of the International Physical Activity Questionnaire (IPAQ) was used to record levels of physical activity: (1) vigorous-intensity activity, (2) moderate-intensity activity, (3) walking and (4) sitting. A combined total physical activity score was calculated and expressed in MET-min/week (Craig et al. 2003). The EPIC Food Frequency Questionnaire (FFQ) was administered at baseline and the FETA software used to extract dietary (energy and nutrient) information (Mulligan et al. 2014).

Blood and urine collection

Fasting blood samples were collected at the beginning of each visit and centrifuged at 3000 rpm for 10 min at 4 °C within 30 min of collection. Aliquots of plasma and serum were frozen and stored at −80 °C for subsequent analyses. Mid-stream urine samples were collected, in fasting conditions, into sterile containers and stored at −20 °C for subsequent analyses.

Biomarker analysis

A modified version of the gas chromatography/mass spectrometry (GC–MS) method proposed by Tsikas (2000) was used to determine NO_3^- and NO_2^- concentrations in urine and plasma samples and sum of NO_3^- and NO_2^- (NO_x) was calculated. However, blood samples were not immediately processed (~30–45 min) to preserve NO_2^- and therefore NO_3^- is the main contributor to the total concentration of NO_x. The protocol and validation of the modified GC–MS method have been described elsewhere (Qadir et al. 2013). Methods for the measurement of glucose, insulin, IL-6, 3-NT, cGMP, ET-1, P-selectin, E-Selectin, intercellular adhesion molecule-3 (ICAM-3) and thrombomodulin are reported in the Online Supplementary Material.

Statistical analysis

Repeated-measures general linear models were used to test the effect of NO_3^- consumption on measures of vascular function and blood biomarkers. Treatment (nitrate vs. placebo) was entered as a group factor (Tr) and the time points of the incremental exercise test as the repeated factor (Ti). Post-hoc comparison between treatment groups at each time point was performed using the Fisher LSD test. Analyses were conducted using Statistica 10 for Windows (StatSoft.Inc, Tulsa, OK). Statistical significance was set at $<.05$.

Results

Participants' characteristics and safety

Twenty participants were randomised to the interventions. One person developed an ischaemic event during the physical exercise testing performed at the second visit and he was excluded from the study (Figure 1). The remaining 19 participants (mean age 64.7 ± 3.0 years) reported no side effects apart for the expected urine discolouration related to the excretion of beetroot juice pigment (beeturia). Baseline VO_2 max of participants was 23.6 ± 5.8 ml/kg/min for men and 20.5 ± 2.8 ml/kg/min for women.

Body weight, dietary intake and self-reported physical activity

Mean baseline BMI was 25.6 ± 3.4 kg/m^2. Body weight did not change during the study in either groups ($p = .51$) (Table S1 of the Online Supplementary Material). Changes in self-reported physical activity were again not different between the placebo and the NO_3^- arms ($p = .99$) (Table S1 of the Online Supplementary Material).

Resting clinic and daily blood pressure

Baseline resting systolic and diastolic BP were 127.4 ± 16.1 mmHg (range: 100.0–168.0 mmHg) and 76.2 ± 9.6 mmHg (range: 61.6–95.7 mmHg), respectively. Clinic systolic BP were not significant after NO_3^- consumption compared to placebo (−5.05 ± 9.45 vs. −2.64 ± 9.04 mmHg, respectively, $p = .42$) (Figure 2(A)). Similarly, daily BP was not significant for both systolic ($p = .75$) and diastolic ($p = .63$) readings measured over the 7-d period (Figure 2(B)).

Augmentation index

NO_3^- consumption did not have a significant effect on AIx ($p = .87$, Figure S3).

Blood pressure and cardiac function during standardised exercise

NO_3^- consumption did not influence systolic BP response ($p = .92$, Figure 3(A)) during exercise, whereas a non-significant trend for lower diastolic BP after NO_3^- supplementation ($p = .08$, Figure 3(B)). Specifically, lower diastolic BP readings were recorded during moderate sub-maximal exercise intensities (work rate: 40 W, 60 W and 80 W). Dietary NO_3^- consumption did not modify parameters of cardiac function (CO, HR, SV and CI) measured at rest, during exercise and post-exercise recovery (Figure 4(A–D)). In addition, 1 week dietary NO_3^- consumption did not modify the association between CO and oxygen consumption (VO_2) (nitrate, B ± SE = 6.04 ± 0.34, $R^2 = 0.60$, $p < .001$; placebo, B ± SE = 6.68 ± 0.42, $R^2 = 0.55$, $p < .001$) measured at different levels of exercise intensities (Figure S4 of the Online Supplementary Material).

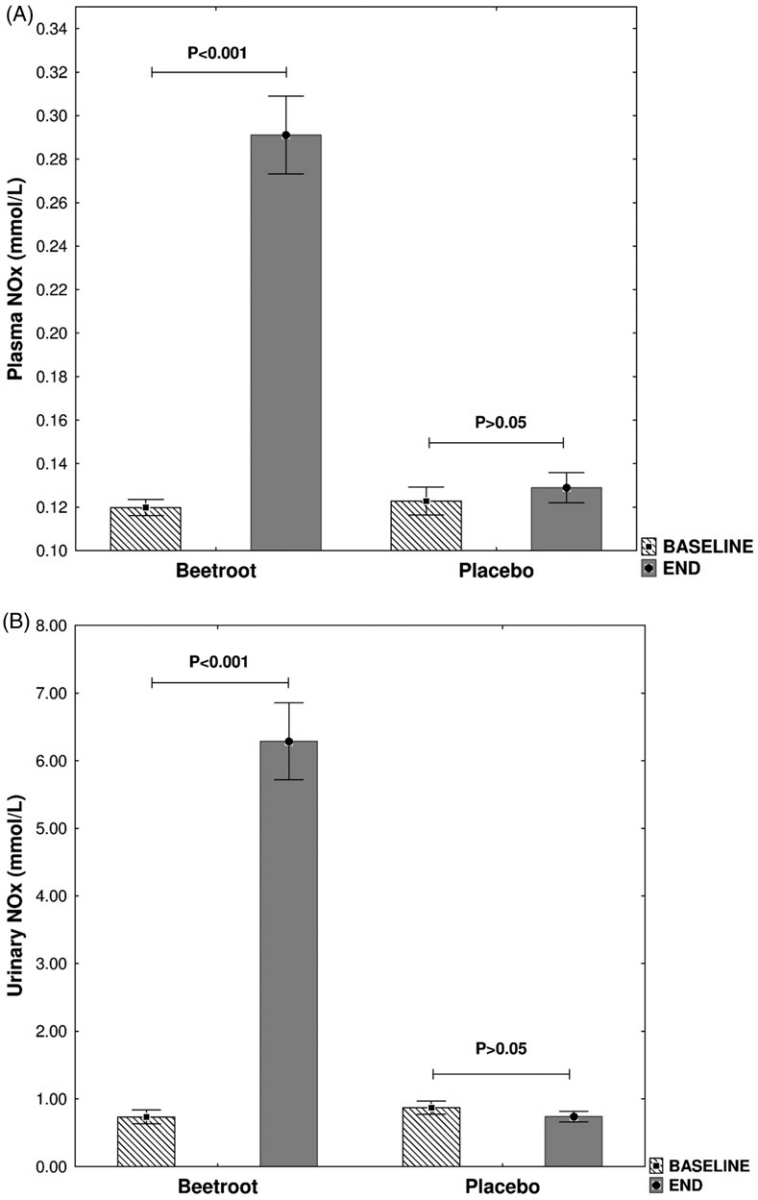

Figure 1. Plasma and urinary nitrate after either 7-d consumption of nitrate-rich or nitrate-depleted beetroot juice. Data presented as mean ±1SE.

Laboratory biomarkers

Concentrations of nitrite plus nitrate ($NO_2^- + NO_3^-$, NO_x) in plasma and urine increased after NO_3^- consumption by 150 ± 77% and 979 ± 488% compared to placebo (−9 ± 33% and −13 ± 34%, respectively). NO_3^- consumption did not modify concentrations of fasting glucose ($p = .41$), insulin ($p = .95$) and HOMA-IR ($p = .88$). NO_3^- consumption also did not induce any changes in biomarkers of endothelial function (cGMP, endothelin-1, E-Selectin, P-Selectin, thrombomodulin and ICAM-3), inflammation (IL-6) and oxidative stress (3-NT) (Table 1).

Discussion

This study does not support a beneficial effect in the short-term of dietary NO_3^- ingestion on cardiac and peripheral vascular health in older healthy adults. In particular, a lack of effect was observed for BP and central haemodynamic responses measured both at rest and during increased metabolic demands. These physiological measurements were complemented by a panel of circulating biomarkers of metabolic control, oxidative stress and endothelial integrity. None of these measurements were altered by 1-week dietary NO_3^- ingestion, which stimulate further discussion

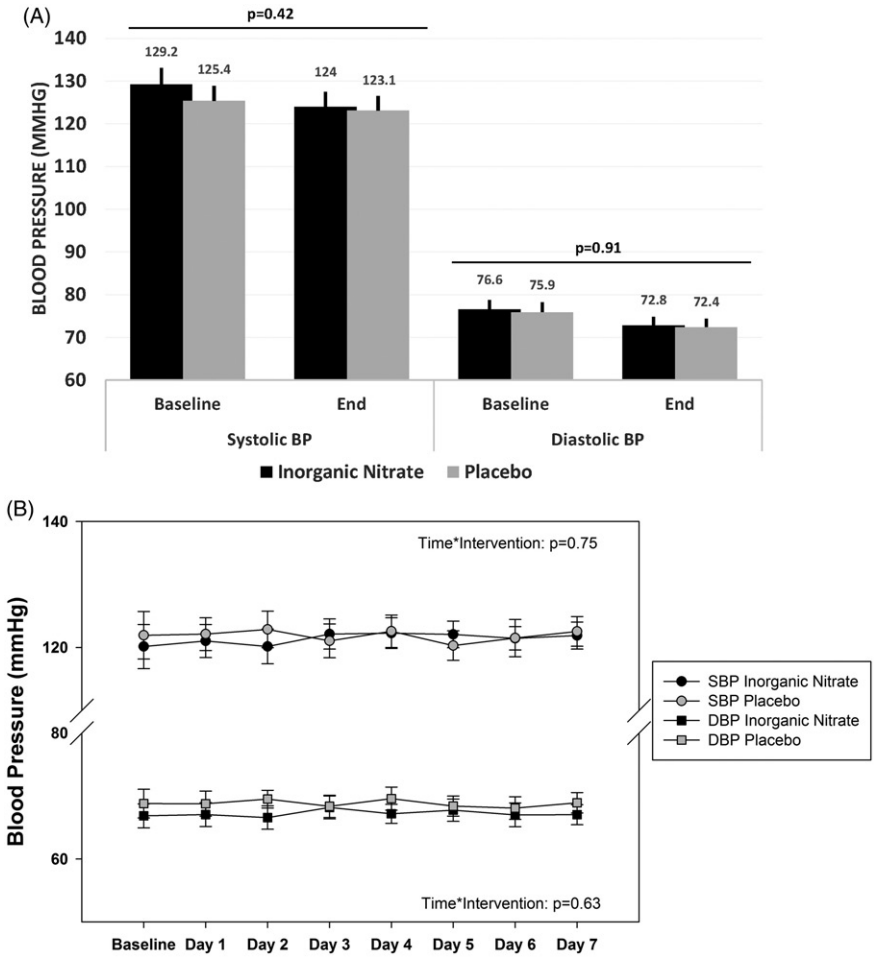

Figure 2. Resting (Panel A) and daily (Panel B) systolic and diastolic blood pressure (BP) measured during a 1 week oral consumption (End) with either nitrate-rich or nitrate-depleted beetroot juice. Data presented as mean ± SEM. SBP: systolic blood pressure; DBP: diastolic blood pressure.

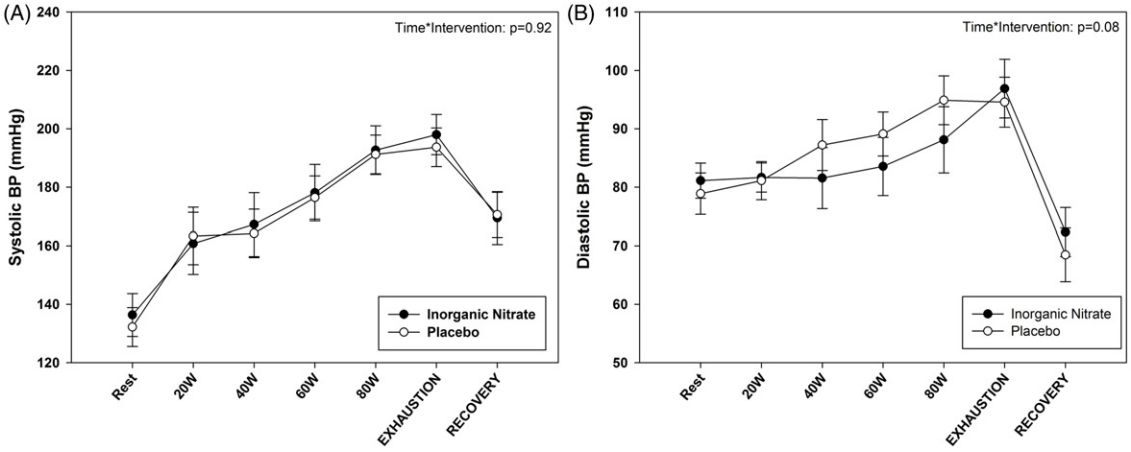

Figure 3. Systolic (Panel A) and diastolic (Panel B) blood pressure (BP) at rest, during incremental exercise and post-exercise recovery after 1 week oral consumption with either nitrate-rich or nitrate-depleted beetroot juice. Data presented as mean ± SEM.

on uncovering the factors that may explain the lack of efficacy in older populations and the contrast with the more consistent beneficial effects observed in younger populations.

Extensive work has been conducted in the last decade to test the effects of dietary NO_3^- on BP but, despite numerous trials, the evidence is still limited due to the small sample size and short duration of

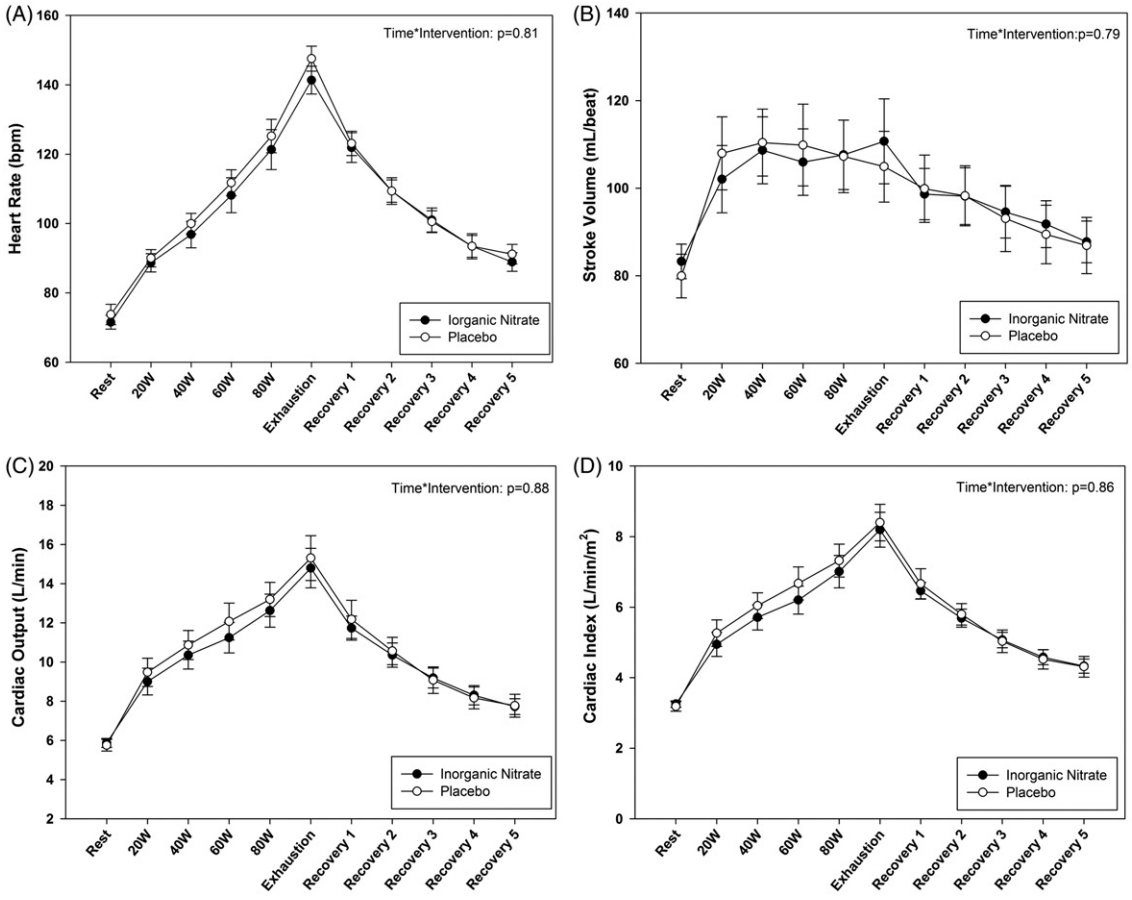

Figure 4. Heart rate (Panel A), stroke volume (Panel B), cardiac output (Panel C) and cardiac index (Panel D) at rest, during incremental exercise and post-exercise recovery after 1 week oral consumption with either nitrate-rich or nitrate-depleted beetroot juice. Data presented as mean ± SEM.

Table 1. Changes in metabolic and vascular biomarkers before and after consumption with either nitrate-rich or nitrate-depleted (placebo) beetroot juice for 1 week.

	Placebo		Inorganic nitrate		
	Baseline	End	Baseline	End	Main effect
Glucose (mmol/l)	5.58 ± 0.61	5.46 ± 0.35	5.38 ± 0.45	5.43 ± 0.35	0.41
Insulin (pmol/l)	34.80 ± 22.20	33.40 ± 19.20	35.32 ± 21.26	33.36 ± 15.45	0.95
HOMA-IR	1.47 ± 0.97	1.36 ± 0.70	1.41 ± 0.93	1.36 ± 0.81	0.88
Interleukin-6 (pg/ml)	3.24 ± 7.24	3.08 ± 5.48	2.80 ± 5.66	3.13 ± 7.54	0.47
cGMP (pmol/ml)	33.27 ± 13.71	35.01 ± 12.02	36.28 ± 13.33	33.90 ± 15.95	0.30
Endothelin-1 (pmol/ml)	1.32 ± 0.02	1.37 ± 0.24	1.39 ± 0.28	1.39 ± 0.31	0.59
E-Selectin (pg/ml)	25.26 ± 12.49	24.42 ± 13.18	29.37 ± 9.65	28.76 ± 8.66	0.96
P-Selectin (pg/ml)	68.75 ± 33.30	62.94 ± 34.88	78.57 ± 24.77	81.98 ± 23.74	0.49
Thrombomodulin (ng/ml)	12.92 ± 5.93	12.03 ± 6.37	15.18 ± 4.04	14.64 ± 3.87	0.89
ICAM-3 (pg/ml)	5.80 ± 2.69	5.47 ± 2.97	6.67 ± 2.11	6.49 ± 2.28	0.89
3-NT (ng/ml)	356.44 ± 360.03	294.24 ± 349.94	208.10 ± 259.54	216.01 ± 201.44	0.37

Data presented as mean ± SD. HOMA-IR: homeostatic model for the assessment of insulin resistance; cGMP: cyclic guanosine monophosphate; ICAM-3: intercellular adhesion molecule 3; 3-NT: 3 nitro-tyrosine.

completed trials (Siervo et al. 2013; Gee & Ahluwalia 2016; Khatri et al. 2016; Mills et al. 2017). Further research is especially needed in older populations, although patients with comorbidities such as peripheral arterial disease or heart failure (HF) appear to receive greater health benefits from dietary NO_3^- consumption (Kenjale et al. 2011; Zamani et al. 2015; Eggebeen et al. 2016). However, DeVan et al. (2015) have recently reported that sodium nitrite supplementation was well-tolerated and improved endothelial function and lessens carotid artery stiffening in middle-aged and older adults. Conversely, our group has recently reported non-significant effects of dietary NO_3^- consumption on endothelial function and on

24-h ambulatory BP in older adults with and without type 2 diabetes (>60 years) (Lara, Ogbonmwan, et al. 2015; Siervo et al. 2015). These results have also recently been corroborated by Bondonno et al. who found no effect of 7-d dietary NO_3^- consumption on home and 24-h ambulatory BP in patients with raised BP (age range: 30–70 years) (Bondonno et al. 2014, 2015). However, Kapil et al. showed that a 4-week intervention in drug-naïve hypertensive subjects (age range: 18–85 years) significantly reduced clinic, home and 24-h ambulatory BP and improved endothelial function (Kapil et al. 2015). The divergence of results is again part of the discussion around the effects of dietary NO_3^- on health outcomes and priority is now being assigned to the identification of factors accounting for the mixed findings. These factors may include phenotypic characteristics (i.e. age, BMI, health status) of the populations, dose of dietary nitrate and duration of supplementation, study design, measurement protocols of BP an vascular health, type of cardiopulmonary fitness test protocols. The dynamic BP responses during exercise have been investigated in young, healthy populations (Bond et al. 2014; Lee et al. 2015) and in patients with COPD (Berry et al. 2015) and HF (Coggan et al. 2015; Coggan & Peterson 2016; Eggebeen et al. 2016). In healthy young populations, two studies reported a decline of sub-maximal systolic BP after acute (single dose) and short-term (15 d) dietary NO_3^- consumption (Bond et al. 2014; Lee et al. 2015). In older COPD patients, dietary NO_3^- decreased systolic BP at rest, whereas only diastolic BP showed a significant decline during 5 W pedalling and 75% of maximal work rate (Berry et al. 2015). In patients with HF, acute dietary NO_3^- did not improve BP responses during knee extension or cycle ergometry tests at sub-maximal and maximal efforts (Coggan & Peterson 2016). However, 1 week of daily dosing with dietary NO_3^- significantly improved submaximal BP in elderly patients with HF with preserved ejection fraction (Eggebeen et al. 2016). Our study is the first trial to investigate resting and dynamic BP responses in older healthy adults and our results do not support a beneficial effect of dietaryNO_3^- on oxygen consumption (data not shown) as well as vascular responses during exercise. Precisely why there is a lack of response to dietary NO_3^- in our study is not known, since we have supplemented subjects for 1 week and administered a NO_3^- dose considerably higher compared to other studies (>700 mg/d). The factors explaining these divergent age-dependent responses are still largely undetermined, which could potentially be related to a decline in the reducing capacity to convert NO_3^- into NO_2^- or sensitivity of cellular targets to NO.

In mice, NO_3^- consumption has been shown to increase the expression of calcium handling proteins in the heart, resulting in increased cardiomyocyte calcium signalling and improved LV contractile function (Pironti et al. 2016). These findings have provided preliminary support to the role of dietary NO_3^- as a cardiac modulator, which have then been translated into clinical interventions in populations without and with impaired cardiac function. In healthy populations, acute dietary NO_3^- consumption did not modify resting or sub-maximal CO (Bond et al. 2014), whereas an improvement of CO and SV was observed after a 1-week dietary NO_3^- consumption (Lee et al. 2015). The only study testing the acute effects of dietary NO_3^- on cardiac function in HF patients with preserved ejection fraction found greater reductions in systemic vascular resistance, aortic AIx and increased CO during exercise (Zamani et al. 2015). We tested whether dietary NO_3^- could minimise the age-related decline in myocardial contractility and ejection fraction, which could prompt compensatory myocardial cardiac hypertrophy (North & Sinclair 2012). This enhances in the short-term CO but the long-term effect of LV hypertrophy is known as represents an important step in the development of HF and coronary syndromes (Gosse 2005). Our scope was to evaluate whether dietary NO_3^- could represent an effective and simple nutritional intervention that may minimise age-related changes in cardiac function and impact, from a primary prevention perspective, on the risk for HF. However, these preliminary results may not support the beneficial effects of dietary NO_3^- on cardiac function in older healthy populations but, if confirmed in more robust trials, dietary NO_3^- may still represent a promising nutritional strategy in patients with impaired cardiac function.

The small sample size and the short duration are important limitations of this trial and a cautious interpretation of the results is needed; nevertheless, our study is to date one of the longest trials testing the effects of dietary NO_3^- on resting and exercise vascular responses in older participants. We did not assess daily dietary intake during the trial. However, participants were asked to maintain their habitual dietary intake during the study and the differences in nitrate intake between intervention and placebo groups were clearly demonstrated by the large differences in plasma and urinary nitrate concentrations. Plasma NO_2^- concentrations were not measured since it was not possible due to logistic constraints to process the samples immediately after collection to minimise

the immediate NO_2^- degradation (half-life: ~5 min). However, in previous studies testing the effects of dietary NO_3^- consumption in older participants where plasma NO_2^- concentration was measured, an increase in plasma NO_3^- and NO_x concentrations similar to the amount observed in this study occurred alongside a significant rise in plasma NO_2^- concentrations (Gilchrist et al. 2013). In addition, the measurement of NO_x was critical to assess the compliance to the interventions as well as the attainment of a significant rise in plasma NO_3^- to enable an increased NO generation via the NO non-enzymatic pathway. Finally, the limitations of bio-reactance for the assessment of cardiac haemodynamic profiles have to be taken into account for the interpretation of the results.

Testing the efficacy of dietary NO_3^- consumption on cardiovascular health is an attractive research area due to the potential use of natural products to increase NO_3^- intake and applicability in long-term dosing. However, this short-term intervention showed that dietary NO_3^- consumption did not modify physiological and biochemical markers of vascular health in healthy older adults. However, these findings are preliminary and require corroboration in studies with longer duration and larger samples of healthy older individuals as well as in older patients with increased cardiovascular risk.

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Disclosure statement

The authors have no conflict of interest to declare.

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