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Ageing modifies the effects of beetroot juice supplementation on 24-hour blood pressure variability: An individual participant meta-analysis

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ABSTRACT

Objectives: Abnormal circadian oscillations of blood pressure (BP) and nocturnal–diurnal BP differences (i.e., dipping) increase cardiovascular risk. Whether inorganic nitrate supplementation influences 24-hr BP variability is currently unknown. We studied the effects of high-nitrate beetroot juice supplementation on BP variability measured by 24-hr ambulatory BP monitoring (24-hr ABPM) in older subjects.
Methods: Data from four independent randomised clinical trials were collated. Eighty-five older participants (age range: 55–76 years) were included in the final database. Two trials had an open-label, parallel design and two trials had a cross-over, double-blind design. Participants were randomised to either beetroot juice or placebo. Changes in 24-hr ABPM (daily, diurnal, nocturnal), variability (weighted-SDs), night-dipping, morning surge for systolic and diastolic BP were measured. Meta-analysis was conducted to obtain pooled estimates of the effect size for each BP outcome. Sub-group analyses were conducted to evaluate the influence of age, BMI, gender, BP status and changes in nitrite concentrations on the effect size.
Results: The pooled effect of beetroot juice on all BP outcomes was not significant. Beetroot juice ingestion determined a significant decrease in nocturnal systolic BP variability in subjects aged less than 65 y (2.8 mmHg, –4.5 –1.0, p = 0.002) compared to the older group (≥65 y; 1.0 mmHg, –2.2 4.2, p = 0.54). A greater change in NO_2^- concentrations after beetroot supplementation was associated with significant differences for nocturnal mean (–3.4 mmHg, –0.6 –2.4, p = 0.02) and variability (–0.8 mmHg, –1.5 –0.06, p = 0.03) of systolic BP.
Conclusions: The vascular responsiveness to inorganic nitrate may be modified by mechanisms of vascular ageing influencing the reducing capacity to convert inorganic nitrate into nitrite and tissue-specific responses to dietary nitrate supplementation.

1. Introduction

Raised blood pressure (BP) is a leading cause of cardiovascular diseases and main contributor to the global burden of non-communicable diseases [1]. The haemodynamic effects of raised BP are responsible for the remodelling of cardiac ventricles [2] and intima-media thickening [3], which increase the risk of cardiovascular diseases such as heart failure or stroke [4]. There is a continuum of cardiovascular risk that increases as BP rises, and the theoretical minimum threshold of risk associated with systolic BP has been estimated to be approximately 115 mmHg [5]. For each 2 mmHg rise in systolic BP there is a 7% increased risk of mortality from ischaemic heart disease and a 10% increased risk of mortality from stroke [6]. These statistics emphasise the importance of small reductions in BP for the effective management and prevention of hypertension-related comorbidities.

Effective nutritional and lifestyle interventions are key to prevent hypertension and related cardiovascular complications [7]. The reduction of salt intake is an example of a nutritional intervention with immediate benefits on BP regulation [8]. More recently,

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inorganic nitrate (NO_3^-) supplementation has been advanced as a potential, effective nutritional strategy to control BP [9,10]. A recent meta-analysis showed a decrease in resting systolic BP of 4.4 mmHg after either inorganic NO_3^- or beetroot juice supplementation [11]. In addition, dietary patterns rich in inorganic NO_3^-, such as the Dietary Approach to Stop Hypertension (DASH diet), have been associated with a reduction in resting systolic and diastolic BP of 5.2 mmHg and 2.2 mmHg, respectively [12].

Twenty-four hour ambulatory BP monitoring (24-hr ABPM) is a reference method for the diagnostic assessment of hypertension and monitoring of anti-hypertensive treatments [13,14]. This method provides information on circadian BP rhythm such as mean diurnal and nocturnal BP, BP variability, night dipping and morning surge [15]. Abnormal values of any of these indexes are independently associated with a greater haemodynamic load and CVD risk [16]. The effects of inorganic NO_3^- supplementation on measures of 24-hr BP variability have not been investigated. We hypothesised that inorganic NO_3^- supplementation may increase nitric oxide (NO) bioavailability [9], via an NO-synthase independent NO generation, and influence both mean values and variability of systolic BP. We also predicted that these effects were more significant on nocturnal BP, which may be explained by the diminution of the putative, confounding effects of physical activity and mental stress on BP regulation.

To address these hypotheses, we collated 24-hr ABPM data originally collected in four independent randomised clinical trials testing the effects of beetroot juice supplementation, as a rich source of inorganic NO_3^-, for a minimum of one week on 24-hr ABPM variability, diurnal and nocturnal BP, night dipping, morning surge and ambulatory arterial stiffness index (AASI) in older subjects (≥55 years). The individual data included in each trial were entered in a meta-analytical model to calculate the pooled effect size for each 24-ABPM systolic and diastolic outcome. Finally, we investigated whether the efficacy of inorganic NO_3^- on 24-hr ABPM outcomes was modified by ageing, gender, obesity, high BP and magnitude of post-supplementation rise in nitrite (NO_2^-) concentrations.

2. Methods

2.1. Study design

All trials were conducted in the UK and recruited older men and women aged 55 years and older. A description of the trial protocols has been previously reported for Trial-1 [17] and Trial-3 [18]. A description of the protocols of Trial-2 and Trial-4 is provided in the Online Supplementary Material. Briefly, two trials were conducted in Newcastle upon Tyne (Newcastle University, Trial-1, Trial-2) and two trials were conducted in Exeter (Exeter University, Trial-3, Trial-4). Both Newcastle-led trials had a two-arm, parallel study design. Trial-1 had a duration of three weeks and included blackcurrant juice as control (200 ml/day) and supplementation of 70 ml/day of concentrated beetroot juice (~4.5 mmol nitrate/day). Trial-2 had a duration of one week and included a negative control (diet only) and supplementation of 140 ml/day (~9.0 mmol nitrate/day) of concentrated beetroot juice. Both Exeter-led trials had a cross-over, double-blind, placebo controlled study design. Trials 3 and 4 had both a duration of two weeks and included alternate, random supplementation of 250 ml/day of NO_3^--rich beetroot juice (7.5 mmol nitrate/day active) or 250 ml/day of NO_3^--depleted beetroot juice (0.002 mmol nitrate/day, placebo). All beetroot juice supplements were provided by the same company (James White Drinks Ltd., UK).

2.2. Subjects

A total of 85 non-smoking men and women (M/F: 50/35), aged 55–76 years old with body mass index (BMI) between 20.2 and 39.5 kg/m^2 were recruited at the Newcastle and Exeter research centres. Trial-1 was approved by the Newcastle University – Faculty of Medical Sciences Ethics Committee (Application No. 00628/2013). Trial-2 was approved by the North East – Northern & Yorkshire Research Ethics Committee (Study No. 12/NE/0134). Trials 3 and 4 were both approved by the Devon and Torbay Research Ethics Committee (Study No. 09/H0202/43). Written informed consent was obtained from all participants prior to participation in each trial.

2.3. Study protocol

Newcastle: A telephone screening interview was conducted to ensure eligibility of participants. Participants attended the research facilities at Newcastle University in fasting conditions. Anthropometric measurements (weight, height and waist circumference) were performed and body mass index (BMI) calculated. Participants were then randomised to one of two interventions (Trial 1: beetroot or blackcurrant juice; Trial 2: beetroot or diet only) and baseline measurements including resting BP, collection of saliva (Trial-1) and plasma (Trial-2) samples, and completion of the International Physical Activity Questionnaire (IPAQ) for the assessment of physical activity. At the end of the visit participants were fitted with a 24-hour AMBP monitor to continuously assess BP over the next 24-hour period. Saliva samples were transferred to a –20 °C freezer within 2 hours of collection. Fasting blood samples were collected in lithium heparin tubes and centrifuged within 30 min from collection. Plasma samples were then immediately transferred to a –80 °C freezer.

The intervention phase started immediately after the completion of the 24-hour BP monitoring period and lasted for 21 and 7 days for Trial-1 and Trial-2, respectively. During this phase, each participant was expected to comply with the assigned nutritional intervention and dietary plan to standardise NO_3^- intake. At the end of the intervention, participants returned to the research unit to repeat the same set of measurements performed at baseline.

Exeter: Subjects were recruited from the Exeter 10000 (EXTEND) bio-resource [18]. Eligible participants were randomised to begin, in either order, a 2-week period of supplementation with 250 ml of beetroot juice daily or 250 ml of NO_3^--depleted beetroot juice, followed by a 4-week washout period before entering the second arm of the study. Subjects were instructed to consume the juice along with their evening meal to minimise any potential glycaemic excursion, typically between 1800 and 2000 hours. Participants continued their usual antihypertensive medication and their usual hypoglycaemic medications including metformin. Hypoglycaemic agents were omitted on visits for which subjects were fasted. Twenty-four-hour blood pressure monitoring was performed from 0900 on day 13 of each supplementation arm. Fasting blood samples for NO_3^- and NO_2^- were collected into lithium heparin collection tubes. Samples were centrifuged immediately and plasma was immediately separated and flash-frozen in liquid nitrogen before transfer to a –80 °C freezer.

2.4. Nutritional supplementation

Newcastle: Participants enrolled in Trial-1 and randomised to the intervention group were asked to drink 70 ml of concentrated beetroot juice (Beet-It Sport Shot, James White Company Ltd, Ipswich UK, 71 kcal) every morning. Each bottle (70 ml) provides approximately 300–400 mg of inorganic NO_3^-. Participants randomised to the control group were asked to drink 200 ml of blackcurrant juice (Capri-Sun Blackcurrant Juice, 100 kcal), 2.7 ± 0.1 mg NO_3^- per bottle every morning. Participants enrolled in Trial-2 and randomised to the intervention group were asked to drink 70 ml of concentrated beetroot juice in the morning and 70 ml in the evening. Participants randomised to the control group were asked to follow the diet