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Nitrate-rich vegetables do not lower blood pressure in individuals with mildly elevated blood pressure: a 4-week randomised controlled crossover trial

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Short running head: Nitrate-rich vegetables and blood pressure.

Abbreviations: NO, nitric oxide; PWA, pulse wave analysis; PWV, pulse wave velocity; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

ABSTRACT

Background: Emerging evidence suggests that increasing intakes of nitrate-rich vegetables may be an effective approach to reducing blood pressure.

Objective: Our primary aim was to determine whether daily consumption of nitrate-rich vegetables over 4 weeks would result in lower blood pressure.

Design: Thirty participants with pre-hypertension or untreated grade 1 hypertension were recruited to a randomised, controlled, crossover trial with 4-week treatment periods separated by 4-week washout periods. Participants completed three treatments in random order: (1) increased intake (~200 g/d) of nitrate-rich vegetables (high nitrate, HN, ~150 mg/d nitrate); (2) increased intake (~200 g/d) of nitrate-poor vegetables (low nitrate, LN, ~22 mg/d nitrate); and (3) no increase in vegetables (control, C, ~6 mg/d nitrate). Compliance was assessed using food diaries and by measuring plasma nitrate and carotenoids. Nitrate metabolism was assessed using plasma, salivary, and urinary nitrate and nitrite concentrations. The primary outcome was blood pressure assessed using 24-hour ambulatory, home, and clinic measurements. Secondary outcomes included measures of arterial stiffness.

Results: Plasma nitrate and nitrite concentrations were increased with the HN treatment in comparison to the LN and control treatments ($P<0.001$). Plasma carotenoids were increased with the HN and LN treatments compared to the control ($P<0.01$). High nitrate treatment did not reduce systolic blood pressure (24-hour ambulatory: HN 127.4±1.1 mmHg, LN 128.6±1.1 mmHg, C 126.2±1.1 mmHg, $P=0.20$; home: HN 127.4±0.7 mmHg, LN 128.7±0.7 mmHg, C 128.3±0.7 mmHg, $P=0.36$; clinic: HN 128.4±1. 3mmHg, LN 130.3±1.3 mmHg, C 129.8±1.3 mmHg, $P=0.49$) or diastolic blood pressure compared with LN and C treatments ($P>0.05$) after adjustment for pre-treatment values, treatment period and treatment order. Similarly, no differences were observed between treatments for arterial stiffness measures ($P>0.05$).

Conclusion: Increased intake of nitrate-rich vegetables did not lower blood pressure in pre-hypertensive or untreated grade 1 hypertensive individuals when compared with increased intake of nitrate-poor vegetables and no increase in vegetables.

Clinical trial registry number and website: This trial was prospectively registered at www.anzctr.org.au as ACTRN12615000194561.

Keywords: blood pressure, vascular stiffness, nitrate, vegetables, hypertension

INTRODUCTION

High blood pressure is the leading risk factor for global disease burden (1). The maintenance of a healthy blood pressure and the prevention of hypertension continues to be a public health priority worldwide (2, 3). It is estimated that for every 10 mmHg reduction in systolic blood pressure there is a 13-28% lower risk of cardiovascular disease events and all-cause mortality (4). Arterial stiffness, which is closely related to hypertension (5, 6), is also a predictor of cardiovascular and all-cause mortality (7). Diets rich in vegetables, such as the vegetarian diet (8), Mediterranean diet, Nordic diet, and Dietary Approaches to Stop Hypertension (DASH) diet (9), have been shown to lower blood pressure. There are many components of a diet rich in vegetables that may benefit cardiovascular health. In particular, emerging evidence suggests that inorganic nitrate found in vegetables can contribute to lowering blood pressure (10, 11).

Inorganic nitrate is a precursor for nitric oxide (NO) (12). Nitric oxide is an important cell signalling molecule and potent vasodilator critical for vascular health (13). The enterosalivary nitrate-nitrite-NO pathway generates NO via the sequential reduction of the anions nitrate and nitrite. This is facilitated by facultative anaerobic bacteria in the oral cavity, the acidic environment of the stomach and numerous endogenous molecules identified as having nitrite reducing ability (14). Increased scavenging of NO and dysfunction of the L-arginine-NO pathway underlie the reduced bioavailability of NO observed in hypertensive individuals (15). Increasing plasma nitrate through dietary means may provide a new therapeutic measure for restoring NO levels.

Vegetables account for approximately 80% of total nitrate consumed in the general population, with the majority coming from leafy green vegetables and beetroot (12, 16). There is now consistent and convincing evidence that an increase in nitrate salts and nitrate-rich vegetables can result in a decrease in blood pressure within hours of ingestion (10, 11, 17-19).

However, data from short-term trials are yet to provide a clear understanding of the effects of a chronic increase in nitrate intake on blood pressure (10). In particular, there is a need to determine whether increasing nitrate-rich vegetables results in sustained lower blood pressure in individuals with elevated blood pressure. The results of published studies in this population are inconsistent (19, 20).

The objective of this study was to investigate whether increased intake of nitrate-rich vegetables would result in lower blood pressure and improved arterial stiffness. An increased intake of nitrate-rich vegetables was compared with a matching increase in nitrate-poor vegetables, and no increase in vegetables.

METHODS

Ethics

The Vegetable Intake and Blood Pressure (VIABP) study (registered at www.anzctr.org.au as ACTRN12615000194561) was approved by the University of Western Australia Human Research Ethics Committee and was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Trial design

The VIABP study was a randomised, controlled, crossover trial with three 4-week treatment periods, each preceded by a 4-week washout period (**Supplemental Figure 1**). The study was conducted at the Royal Perth Hospital Medical Research Foundation, Perth, Australia. Participants were randomly assigned to one of six sequence orders for the three treatments using computer-generated random numbers, assigned upon randomisation. The three treatments were: (1) increased intake of nitrate-rich vegetables (high nitrate diet, ~200 g/d); (2) increased intake of nitrate-poor vegetables (low nitrate diet, ~200 g/d); and (3) no increase in vegetables (control diet). Throughout the entire 24-week trial period all participants were asked to limit their intake of nitrate-rich vegetables, except whilst undertaking the high nitrate treatment (**Supplemental Table 1**).

Participants

Thirty participants with pre-hypertension or untreated grade 1 hypertension were recruited from the Perth general population using newspaper advertisements between March and July 2015. Participants were ambulant men and women between the ages of 21 and 75 years, with a resting mean systolic blood pressure between 120-160 mmHg, inclusive. Participants were excluded if they were: diabetic; a smoker; taking antihypertensive medication, nitric oxide donors, organic nitrates and nitrites, or related drugs; had a body mass index (BMI) $\geq$35 kg/m^2 or <18.5 kg/m^2; or used antibacterial mouth wash. For a complete list of inclusion and

exclusion criteria see **Supplemental Table 2**. Participants underwent screening procedures consisting of anthropometric measurements (height, weight, waist circumference, and hip circumference), an electrocardiogram, and a fasting blood test, which consisted of a full blood count, lipid profile, and glucose test, analysed by PathWest laboratories (Royal Perth Hospital, Perth, Australia). Blood pressure was assessed using a Dinamap 1846SX/P oscillometric recorder (Critikon Inc., Tampa, FL, USA). Participants were asked to rest for 5 minutes before five blood pressure and heart rate readings were taken at 2 minute intervals. The first measurement was excluded and the mean of the next four consecutive readings was used to determine resting blood pressure. After blood tests confirmed eligibility, participants were asked to return to the research clinic to complete a medical examination by one of our physicians (IBP and LJB). Participants provided a list of current medications and supplements, and a medical history. At the same visit, participants completed a validated food frequency questionnaire (Dietary Questionnaire for Epidemiological Studies Version 2, DQES v2) (21-23) to obtain baseline dietary intake. Participants were supervised by a nutritionist whilst completing the questionnaire. Food models, food charts, measuring cups, and measuring spoons were provided to ensure the accuracy of reported food consumption.

Dietary interventions

During each 4-week treatment period, additions were made to participants' breakfast and dinner, consisting of vegetables blended into juices or a matching control juice. Participants were asked to maintain all meals as usual with the exception of limiting high nitrate vegetables where possible. In the high and low nitrate treatment periods participants were asked to consume 100 g/d of high and low nitrate vegetables, respectively, before breakfast and dinner. This was equivalent to increasing vegetable intake by approximately 2.7 serves/d (~200-300 kJ/d) (24). For the control treatment, participants were asked to blend a quarter of an orange and 8 g maltodextrin in water (approximately energy matched to the vegetables

consumed in the high and low nitrate treatments). Prior to randomisation, participants consumed a sample juice for all three treatments and completed a juice acceptability questionnaire to ensure willingness to participate. They were provided with blenders and blended at least three different vegetables of approximate equal weight with a quarter of an orange (for taste) and less than 1 cup (~200 ml) of water. For the low nitrate treatment, participants were instructed they could microwave, oven bake, boil, or steam any low nitrate root vegetables before consumption, and were asked not to add any condiments (e.g. cooking oil, salt). The high and low nitrate vegetables consumed by participants are presented in **Supplemental Table 3**. Participants were asked to freeze a small sample of each treatment juice in a sterile 5 ml tube in the week prior to their post-treatment visit. Nitrate and nitrite concentrations were determined for each treatment juice. In addition, participants kept a diary of the type and weight of all vegetables (high nitrate and low nitrate) consumed in all prepared juices. Treatment diaries along with plasma concentrations of nitrate, nitrite, and total carotenoids were used to assess compliance.

Clinical measurements

Anthropometric measurements (body weight, waist circumference, and hip circumference) were assessed pre- and post-treatment. Participants were wearing minimal clothing and no shoes. Standing height (m) was measured without shoes using a wall-mounted stadiometer to the nearest 0.01 m. Fasting body weight (kg) was measured without shoes using electronic scales to the nearest 0.01 kg. Waist and hip circumference (cm) were measured using a steel tap measure (Lufkin Executive Thinline, W606PM, USA). Waist and hip measurements were used to calculate waist-to-hip ratio (waist cm / hip cm).

Physical activity was assessed pre- and post-treatment using the validated short form of the International Physical Activity Questionnaire (IPAQ) (25). Total physical activity was calculated in MET-min/week and then converted to kJ/d. Alcohol intake was assessed pre-

and post-treatment using a 7 day alcohol diary. Standard drinks were calculated by multiplying the volume of alcohol consumed (L), the percentage of alcohol consumed (%) and the specific gravity of ethyl alcohol (0.789). Alcohol was converted to g/d.

Blood pressure

Ambulatory blood pressure

Ambulatory blood pressure and heart rate were monitored every 20 minutes during the day and every 30 minutes overnight for a 24-hour period commencing at the end of pre- and post-treatment visits, as previously described (26). Measurements showing an error code or those with a pulse pressure of less than 20 mmHg were excluded from the analysis. Blood pressure traces that were missing more than four hourly means over the 24 hours were also excluded from the analysis. A minimum of 70% successful readings was considered a valid recording. Mean blood pressure was determined for the 24-hour, daytime (06:00-21:59), and night-time (22:00-05:59) period.

Home blood pressure

Participants were provided with a digital blood pressure monitor (UA-767PC, A&D Co., Ltd, Saitama, Japan) and an appropriately sized upper-arm cuff. Participants were given appropriate training and instructions on its use prior to the study commencing. Home blood pressure was measured and recorded by each participant three times daily (shortly after waking and prior to breakfast, 1-2 hours prior to dinner, and 1-2 hours after dinner) for the entire study duration. Participants were instructed to rest for 5 minutes prior to commencing three blood pressure readings over a 3-minute period. The first measurement was excluded from the analysis and the mean of the second and third measurements used. Mean blood pressure was determined for the overall 4-week pre- and post-treatment periods; week 1, week 2, and week 3 of each treatment; the last 7 days pre- and post-treatment period; and the morning, afternoon, and evening of the last 7 days pre- and post-treatment period.

Clinic blood pressure

Clinic blood pressure was measured pre- and post-treatment using the SphygmoCor XCEL device (2012 AtCor Medical Pty. Ltd., Sydney, Australia). An appropriately sized blood pressure cuff was fitted to each participant's non-dominant arm approximately 2.5 cm above the antecubital fossa. Participants were fasting for at least 12 hours and were asked to rest in a supine position for 5 minutes prior to three blood pressure measurements performed at 60 second intervals. The first blood pressure reading was excluded and the mean of the second and third measurements used in analysis.

Arterial stiffness

Pulse wave analysis (PWA) and pulse wave velocity (PWV) were measured pre- and post-treatment using the SphygmorCor XCEL device (2012 AtCor Medical Pty. Ltd., Sydney Australia). Participants were fasting for at least 12 hours, and avoided vigorous exercise and alcohol intake for at least 24 hours prior to assessment. Participants were asked to rest for 5 minutes in a supine position before three blood pressure measurements at 60 second intervals were taken. Clinic blood pressure was followed by one 10 second capture PWA reading, which included central aortic systolic pressure, central aortic diastolic pressure, and augmentation index (%). An appropriately sized cuff was then fitted to the participants' right thigh. Tubing was attached and a probe placed on the carotid artery. When a pulse was detected the femoral cuff inflated and captured aortic PWV. Two PWV measurements were taken and the average used in the analysis. If a >0.5 m/s difference between the first two measurements was observed, a third measurement was taken and the middle value of the three measurements was used.

Biochemical analyses

Fasting (≥12 hours) blood, saliva, and urine samples were collected pre- and post-treatment. Blood samples were collected by venepuncture into EDTA and Lithium Heparin Plasma with

Gel (LH PST II) tubes. Whole blood was centrifuged at 4^0C, 3500 rpm for 10 minutes and plasma aliquots stored at -80^0C until analysis. Participants spat into sterile polystyrene jars for 5 minutes to obtain saliva samples and saliva aliquots stored at -80°C until analysis. Spot urine samples were also collected into sterile polystyrene jars and aliquots stored at -80°C until analysis.

Nitrate and nitrite analysis

Nitrate and nitrite concentrations were measured in plasma, saliva, urine, and juice samples using gas chromatography/mass spectrometry (GCMS) with ^{15}N-labelled nitrate and nitrite as internal standards, as previously described (27).

Plasma carotenoid analysis

High-performance liquid chromatography (HPLC) methodology was used to determine plasma carotenoid concentrations of β-carotene, lycopene, α-carotene, β-cryptoxanthin, and lutein/ zeaxanthin, as previously described (28). Carotenoids were extracted using ethanol, ethyl acetate, and hexane, with canthaxanthin as an internal standard. Following evaporation of the solvents, the dried extract was reconstituted in dichloromethane: methanol (1:2 /vol) and chromatography performed on an Agilent 1200 HPLC system using Hypersil ODS column (100 mm X 2.1 mm X 5 um). Carotenoids were analysed using a mobile phase of acetonitrile: dichloromethane: methanol 0.05% ammonium acetate (85:10:5 v/v) at a flow rate of 0.3 mL/min, using a diode array detector (450 nm).

Other biochemical analyses

Urinary concentrations of sodium, potassium, and creatinine as well as plasma concentrations of sodium, potassium, creatinine, glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and calculated low-density lipoprotein (LDL) cholesterol were analysed by PathWest laboratories (Fiona Stanley Hospital, Perth, Australia).

Statistics

Sample size

The required sample size for the study was based on the primary outcome of blood pressure measured using 24-hour ambulatory monitoring. It was estimated 25 participants would provide >80% power to detect a 2.0 mmHg difference in mean 24-hour systolic blood pressure, based on data from our previous studies (29, 30). This calculation assumes a type I error rate of 0.05/3 (0.017). In addition, we had >80% power to detect a 2.0 mmHg difference in mean home systolic blood pressure, based on data from our previous studies (29, 30).

Statistical methods

Global statistical significance was set at a 2-sided Type 1 error rate of $P<0.05$. All data were analysed using IBM SPSS Statistics for Windows, version 21.0 (IBM) and SAS software, version 9.4 (SAS Institute Inc.). Normality of distributions was tested using the Shapiro-Wilk normality test. Descriptive statistics of normally distributed continuous variables were expressed as mean ± standard deviation (SD), non-normally distributed continuous variables were expressed as median and interquartile range, and categorical variables as number and proportion (%). Differences between treatments (high nitrate, low nitrate, and control) were tested for each outcome variable using a repeated measures mixed model (proc mixed command) with additional adjustments for outcome pre-treatment values, treatment period and treatment order. We tested for any carryover effects between treatment periods using a treatment*treatment period interaction term.

RESULTS

Recruitment began on 25 May 2015 and final data was collected on 5 May 2016. Of the 65 participants screened for the study, 32 participants were randomised of whom 30 completed the study. Two participants withdrew after randomisation due to medical reasons unrelated to the trial (**Figure 1**).

Baseline demographic and clinical characteristics for the 30 participants are shown in **Table 1**. Baseline dietary data is shown in **Supplemental Table 4**. At baseline, the mean (SD) total nitrate, estimated using a nitrate content of vegetables database (31) as well as published food composition data, was 84.7 (36.2) mg/d (81.9% from vegetables alone). The mean (SD) total vegetable intake was 181.8 (75.8) g/d. Using participants' food diaries from each treatment period, the median (IQR) reported increase in nitrate-rich vegetables for the high nitrate treatment was 201.5 (198.7-204.1) g/d and the increase in nitrate-poor vegetables for the low nitrate treatment was 201.7 (197.0-207.7) g/d. Descriptive statistics for the individual nitrate-rich and nitrate-poor vegetables consumed in both the high and low nitrate treatments are shown in **Supplemental Table 3**. The median (IQR) nitrate and nitrite concentrations, from the juice samples provided by participants, and in which the participants were estimated to be consuming, were: 149.1 (118.2-237.0) and 3.96 (2.19-5.20) mg/d for the high nitrate treatment (n=30); 21.5 (13.9-29.9) and 0.31 (0.16-0.48) mg/d for the low nitrate treatment (n=27); and 5.5 (3.7-8.0) and 0.06 (0.05-0.08) mg/d for the control (n=29), respectively. Nitrate and nitrite concentrations were significantly different between treatment juices ($P<0.001$ for both). High nitrate juices were 27-fold higher in nitrate and 66-fold higher in nitrite concentration than that of the control ($P<0.001$ for both). In addition, the high nitrate juices were 6.9-fold higher in nitrate and 12.8-fold higher in nitrite concentration than that of the low nitrate juices ($P<0.001$ for both). The low nitrate and control juices were not different in nitrate ($P=0.326$) and nitrite ($P=0.779$) concentrations.

Compliance

Compliance was measured for the three treatments using self-reported food diaries. Participants recorded the weight (g) of each vegetable consumed in their juice on a daily basis. Compliance was calculated by dividing the number of days the vegetables were consumed by the number of days the vegetables should have been consumed and then multiplying by 100. On the basis of reported consumption of treatments using food diaries, the median (IQR) compliance was 98.1 (90.6-100.0%) for the high nitrate treatment, 98.1% (92.9-100.0%) for the low nitrate treatment, and 98.1% (96.0-100.0%) for the control treatment. Compliance for the control diet was also measured by dividing the amount (g) of returned maltodextrin by the amount (g) of maltodextrin administered to participants before their treatment period and then multiplying by 100. On the basis of returned maltodextrin, the median (IQR) compliance was 131.5% (110.7-142.5%). All treatment diets were well tolerated. Whilst on the control treatment, one participant reported constipation symptoms and another reported loose stools. No serious adverse events were reported.

Biomarkers of intake and metabolism

Plasma nitrate and nitrite

Median (IQR) plasma nitrate and nitrite concentrations pre- and post-treatment are shown in **Table 2**. Plasma nitrate was significantly different between treatments (P<0.001) (**Figure 2**). There was a 1.7-fold increase in plasma nitrate concentrations for the high nitrate treatment compared to the low nitrate treatment (P<0.001) and a 1.6-fold increase for the high nitrate treatment compared to control (P<0.001) (Figure 2). Plasma nitrite was significantly different between treatments (P=0.007) (Figure 2). There was a 1.5-fold increase in plasma nitrite concentrations for the high nitrate treatment compared to the low nitrate treatment (P=0.002) and a 1.3-fold increase for the high nitrate treatment compared to control (P=0.037) (Figure 2).

Salivary nitrate and nitrite

Median (IQR) salivary nitrate and nitrite concentrations for pre- and post-treatment are shown in Table 2. Salivary nitrate was significantly different between treatments (P=0.038) (Figure 2). There was a 1.7-fold increase in salivary nitrate concentrations for the high nitrate treatment compared to the low nitrate treatment (P=0.022) and a 1.6-fold increase for the high nitrate treatment compared to control (P=0.036) (Figure 2). Salivary nitrite was not significantly different between treatments (P=0.098) (Figure 2).

Urinary nitrate and nitrite

Median (IQR) urinary nitrate and nitrite concentrations adjusted for urinary creatinine are presented in Table 2. Urinary nitrate was significantly different between treatments (P<0.001) (Figure 2). There was a 1.9-fold increase in urinary nitrate concentration for the high nitrate treatment compared to the low nitrate treatment (P<0.001) and a 1.8-fold increase for the high nitrate treatment compared to the control (P<0.001) (Figure 2). Urinary nitrite was not significantly different between treatments (P=0.074) (Figure 2).

Plasma carotenoids

Descriptive statistics for plasma total carotenoids, lutein, beta-cryptoxanthin, lycopene, alpha-carotene, and beta-carotene are presented in **Table 3**. Plasma total carotenoids were significantly different between treatments (P<0.001) (**Figure 3**). There was a 1.4-fold increase in the high nitrate treatment compared to the control (P<0.001) and a 1.3-fold increase in the low nitrate treatment compared to the control (P=0.002) (Figure 3). Plasma lutein was significantly different between treatments (P<0.001) (Figure 3). There was a 1.6-fold increase in plasma lutein in the high nitrate treatment compared to the low nitrate treatment (P<0.001) and a 1.8-fold increase in the high nitrate treatment compared to the control (P<0.001) (Figure 3). Plasma beta-carotene was significantly different between treatments (P=0.002) (Figure 3). There was a 1.6-fold increase in plasma beta-carotene in the high nitrate treatment compared

to the control (P=0.002) and a 1.6-fold increase in the low nitrate treatment compared to the control (P=0.003) (Figure 3). Plasma beta-cryptoxanthin, lycopene, and alpha-carotene were not significantly different between treatments (Figure 3).

Plasma and urinary sodium and potassium

Plasma and urinary sodium and potassium, and urinary sodium to potassium ratio were not significantly different between treatments (**Table 4**).

Blood pressure

Ambulatory blood pressure

Ambulatory measures of blood pressure were excluded for 2 (6.7%) participants due to equipment malfunction (n=1) and <70% successful readings (n=1). The mean (SD) 24-hour, daytime, and night-time ambulatory measures of blood pressure and heart rate pre- and post-treatment are presented in **Table 5**. There were no significant differences between treatments for mean 24-hour, daytime, or night-time ambulatory blood pressure and heart rate (**Figure 4**). No carryover effects were observed for 24-hour, daytime, and night-time ambulatory measures of blood pressure and heart rate (P>0.05 for all). In a post-hoc sensitivity analysis in which we adjusted for age, gender and BMI, the results were very similar and not substantively changed (data not shown).

Home blood pressure

Home measures of blood pressure were complete for all participants. Mean (SD) home measures of blood pressure and heart rate pre- and post-treatment are presented in Table 5. There were no significant differences between treatments for blood pressure and heart rate for the last 7 days of treatment (Figure 4). There were also no significant differences between treatments for blood pressure and heart rate for week 1, week 2 and week 3 of treatment when analysed separately (**Figure 5**). In addition, we found no significant differences between treatments for blood pressure and heart rate measured in the morning, afternoon, and evening

when analysed separately (data not shown). Mean daily home measures of blood pressure and heart rate are shown in **Supplemental Figure 2**. No carryover effects were observed for home measures of blood pressure and heart rate (P>0.05 for all).

Clinic blood pressure

Clinic measures of blood pressure were complete for all participants. Mean (SD) clinic measures of blood pressure and heart rate pre- and post-treatment are presented in Table 5. There were no significant differences between treatments for blood pressure and heart rate (Figure 4).

Arterial stiffness

Pulse wave analysis was complete for all participants. Pulse wave velocity was incomplete for 4 (13.3%) participants due to an inability to obtain measurements. Mean (SD) central systolic and diastolic pressures, central augmentation index (%), and pulse wave velocity for pre- and post-treatment are shown in **Table 6**. No significant differences were observed between treatments for central systolic blood pressure, central diastolic blood pressure, central augmentation index, and pulse wave velocity.

Plasma lipids and glucose

Plasma total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and glucose were not significantly different between treatments (Table 4).

Anthropometry, physical activity and alcohol intake

Descriptive statistics for anthropometry, physical activity, and alcohol intake pre- and post-treatment are presented in **Supplemental Table 5**. Weight, BMI, waist circumference, hip circumference, and waist-to-hip ratio were not significantly different between treatments. Physical activity expended in kJ/d and alcohol consumed in the last 7 days prior to each treatment period were also not significantly different between treatments.

DISCUSSION

In this 4-week randomised controlled crossover trial, an additional ~200 g/d intake of nitrate-rich vegetables did not alter blood pressure or arterial stiffness in men and women with pre-hypertension or untreated grade 1 hypertension. There was a significant increase in salivary, urinary, and plasma nitrate as well as plasma nitrite concentrations after the high nitrate treatment, confirming the dietary interventions were effective in altering nitrate concentrations. In addition, total plasma carotenoids were increased with increased consumption of both high and low nitrate vegetables. Our study findings did not support our hypothesis that an increased intake of high nitrate vegetables would result in lower blood pressure and improved arterial stiffness.

A number of short-term trials have assessed the effects of increased nitrate intake on blood pressure (10, 11). The results of these studies are inconsistent, with several trials finding lower blood pressure with increased nitrate intake (19, 32-37), and others finding no effect (20, 38-42). Our study is unique in that the design assessed the impact of a sustained 4-week increase in nitrate-rich vegetables in individuals with pre-hypertension or untreated grade 1 hypertension and that subjects were not taking anti-hypertensive medication which could have modified any effect of nitrate. There are many potential factors that could influence whether an increase in nitrate intake results in lower blood pressure. The duration of the study appears to be a factor, with acute studies consistently showing blood pressure lowering effects (17, 42-53). Other factors may include the dose of nitrate provided; the background nitrate and vegetable intake of the study participants, which may alter the effective dose; whether there is an individual threshold level, beyond which there is little additional benefit; and the age and health status of the participants.

Only three studies have investigated the short-term sustained effects of nitrate ingestion on blood pressure and arterial stiffness over a period of four or more weeks (19, 33, 42). Of

these, only one has investigated the effects in pre-hypertensive or untreated hypertensive individuals (19). Kapil et al (19) found daily consumption of beetroot juice (nitrate dose: 398 mg/d or 6.4 mmol/d) reduced blood pressure and improved arterial stiffness in 64 untreated or treated hypertensive individuals. Although our study does not align with the aforementioned study (19), other short-term intervention studies (3-42 days) have demonstrated no effect of nitrate ingestion on blood pressure (20, 38-42) or arterial stiffness (40, 41). These studies were all in individuals at risk for cardiovascular disease (20, 38-42). Previous trials where improvements have been observed in blood pressure and arterial stiffness with increased nitrate intake have been a mix of healthy participants (34-37) and those at risk of cardiovascular disease (19, 32, 33, 42). There is strong evidence to show that nitrate ingestion reduces blood pressure within hours of ingestion (17, 42-53). The acute effects on arterial stiffness are less clear. Some studies demonstrate improvements (42, 44, 53) while others do not (47, 50, 51, 54).

Several factors may explain why our study did not demonstrate a reduction in blood pressure. As mentioned previously, background diet may have influenced nitrate metabolism with individuals having sufficient nitrate intake unresponsive to further nitrate supplementation. Possible interactions between nitrate and sulphur-containing dietary constituents have been proposed (55). Nitrate in drinking water was inversely associated with blood pressure at low sulphate concentrations (9-33 mg/L), but this relationship reversed at medium to high concentrations (34-102 mg/L). Such an interaction would unlikely to have confounded the results of our study as the calculated mean sulphate concentration in water consumed by our cohort was 13.5 mg/L (data not shown). This data, however, does not fully discount such an interaction as sulphur-containing foods within the diet cannot be calculated due to the absence of adequate nutrient databases. In addition, Montenegro et al (56) has recently demonstrated acid suppressing drugs abolished the blood pressure lowering effects of oral nitrite ingestion

despite the increase in plasma nitrite concentration. Only one participant in our study reported taking an acid suppressing drug (Rabeprazole) for the treatment of gout. This does not discount the theory that gastric pH may differ between individuals and that it is a possible determinant in influencing blood pressure after nitrate ingestion.

Limitations to this intervention study need to be considered. Firstly, we did not observe differences in urinary potassium concentrations. We may have expected to see a >10% increase in potassium excretion given the self-reported increases in vegetable intake. This could be the result of using creatinine-adjusted values from a spot urine instead of a 24-hour urine sample to assess urinary potassium (57). We did, however, observe a ~1.6 fold increase in plasma nitrate and nitrite with increased nitrate-rich vegetables and a ~1.4 fold increase in plasma total carotenoids with increased nitrate-rich and nitrate-poor vegetables. The increase in plasma nitrate, nitrite, and total carotenoids gives us confidence the participants were compliant. It should be noted that plasma nitrite concentrations in our study are higher than some reported values in the literature. This could be due to a number of factors; however, it is likely an outcome of the GCMS method compared to gas phase chemiluminescence (27). Secondly, our study demonstrated no statistical difference in salivary and urinary nitrite concentrations. Circulating nitrate and nitrite concentrations depend on when nitrate is last ingested. The half-life of nitrate in plasma is 5-6 hours (58). Nitrite is likely to be similar as new nitrite is being continuously generated from the ingested nitrate. As participants were asked to fast for at least 12 hours prior to providing samples, this may explain why no increases in salivary and urinary nitrite concentrations were observed. However, other mechanisms may explain this observation and warrants further investigation. Thirdly, the estimated nitrate intake from the measured juice samples was 149 mg/d, whereas the estimated nitrate intake using a comprehensive international nitrate database (31) was 326 mg/d. The nitrate content of vegetables available in Perth may be appreciably lower than

478 many other regions of the world due to high intensity of sunlight and longer daylight hours.
479 Vegetables grown in lower light intensity and fewer daylight hours have a tendency to
480 accumulate higher nitrate concentrations (59, 60). Fourthly, due to the nature of the
481 intervention, participants could not be blinded to the treatments received. Blinding was,
482 however, utilised for all laboratory analyses. Lastly, we cannot rule out any small effects on
483 blood pressure, less than approximately 2 mmHg.
484 In summary, our findings suggest no short-term clinically significant effects on blood
485 pressure, arterial stiffness, lipids and glucose from increasing the intake of nitrate-rich
486 vegetables in men and women with pre-hypertension or untreated grade 1 hypertension. There
487 are likely complex issues surrounding why no benefit was seen, including background nitrate
488 intake, the level of increase in nitrate intake, cross-talk mechanisms and populations at risk of
489 cardiovascular disease, which may all play a vital role in the differences observed between
490 studies.

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Table 1. Demographic and clinical characteristics of study participants at screening[1]

	All participants n = 30
Demographics	
Male/female, n	20/10
Age, years	63.0 [55.5-70.5]
BMI, kg/m^2	27.0 ± 3.9
Waist circumference[2], cm	89.5 ± 11.7
Hip circumference[2], cm	102.0 [95.0-104.5]
Waist-to-hip ratio[2]	0.9 ± 0.1
Smoking history[2]	11 (37.9)
Medications	
HMG-CoA reductase inhibitors	5 (16.7)
Clinic blood pressure	
Systolic blood pressure, mmHg	133.6 ± 8.4
Diastolic blood pressure, mmHg	77.7 ± 8.0
Heart rate, bpm	61.6 ± 8.0
Biochemistry	
Total cholesterol, mmol/L	5.5 [4.3-6.3]
Triglycerides, mmol/L	1.2 [0.8-1.7]
LDL cholesterol, mmol/L	3.6 ± 1.2
HDL cholesterol, mmol/L	1.3 ± 0.3
Glucose, mmol/L	5.3 ± 0.4

[1]Results are displayed as mean ± SD, median [IQR] or n (%). BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein.
[2]n=29.

Table 2. Descriptive statistics for nitrate and nitrite concentrations in plasma, saliva and urine by treatment, and the between-treatment differences[1]

	C (n=30)	LN (n=30)	HN (n=30)	Treatment effect[2]	
				HN vs. C	HN vs. LN
Nitrate					
Plasma, µmol/L					
Pre	22.4 [17.5-27.5]	23.5 [18.7-28.3]	23.0 [16.2-32.6]		
Post	22.5 [19.0-30.2]	22.7 [15.2-27.2]	34.3 [26.1-52.1]	15.8 ± 4.1[3]	16.7 ± 4.3[3]
Salivary, µmol/L					
Pre	126.4 [48.5-194.1]	116.1 [35.8-197.3]	94.1 [38.1-152.4]		
Post	107.9 [39.3-214.7]	75.6 [36.1-190.1]	134.8 [33.2-341.6]	91.5 ± 42.6[4]	102.4 ± 43.3[4]
Urinary, µmol/mmol creatinine					
Pre	48.8 [28.6-63.9]	38.2 [28.1-58.0]	42.9 [29.0-62.6]		
Post	47.4 [30.2-73.5]	44.4 [29.7-58.4]	79.4 [47.8-138.8]	39.5 ± 10.7[3]	43.0 ± 11.2[3]
Nitrite					
Plasma, µmol/L					
Pre	2.1 [1.6-2.9]	2.1 [1.6-2.7]	2.3 [1.7-2.7]		
Post	2.4 [1.9-3.2]	2.0 [1.4-2.5]	2.8 [2.2-4.2]	0.7 ± 0.3[4]	1.1 ± 0.3[4]
Salivary, µmol/L					
Pre	53.9 [17.0-112.0]	48.7 [18.9-101.0]	36.8 [15.0-67.8]		
Post	61.0 [25.6-90.9]	41.9 [12.5-92.1]	67.0 [16.1-157.1]	29.5 ± 19.9	42.6 ± 20.0[4]
Urinary, µmol/mmol creatinine					
Pre	6.7 [2.7-16.3]	9.3 [2.9-16.6]	8.2 [3.9-18.0]		
Post	9.1 [4.1-18.0]	13.8 [3.2-22.9]	13.0 [3.5-38.0]	8.6 ± 3.8[4]	6.4 ± 4.0

[1]Results are presented as median [IQR]. n=30. C, control; LN, low nitrate; HN, high nitrate.

[2]Between-treatment differences are for the post-treatment values of the high nitrate (HN) compared with low nitrate (LN) and control (C) treatments, adjusted for pre-treatment values, treatment period and treatment order using repeated measures mixed modelling.

[3]P<0.001.

[4]P<0.05.

Table 3. Descriptive statistics for plasma carotenoid concentrations by treatment, and the between-treatment differences[1]

	C (n=30)	LN (n=30)[2]	HN (n=30)	Treatment effect[3]	
				HN vs. C	HN vs. LN
Total carotenoids, mg/L					
Pre	1.675 [1.200-2.212]	1.711 [1.360-2.457]	1.701 [1.305-2.205]		
Post	1.613 [1.257-2.217]	1.850 [1.524-2.668]	2.131 [1.637-2.560]	0.658 ± 0.170[4]	0.073 ± 0.178
Lutein, mg/L					
Pre	0.316 ± 0.107	0.321 ± 0.114	0.303 ± 0.102		
Post	0.313 ± 0.120	0.355 ± 0.120	0.547 ± 0.176	0.246 ± 0.027[5]	0.202 ± 0.029[5]
Beta-cryptoxanthin, mg/L					
Pre	0.337 [0.269-0.539]	0.387 [0.272-0.506]	0.361 [0.249-0.551]		
Post	0.385 [0.292-0.504]	0.406 [0.310-0.736]	0.362 [0.306-0.547]	-0.067 ± 0.047	-0.098 ± 0.049
Lycopene, mg/L					
Pre	0.036 [0.018-0.067]	0.041 [0.023-0.081]	0.042 [0.021-0.075]		
Post	0.033 [0.023-0.065]	0.052 [0.018-0.084]	0.037 [0.022-0.069]	-0.002 ± 0.005	0.010 ± 0.006
Alpha-carotene, mg/L					
Pre	0.047 [0.027-0.076]	0.047 [0.024-0.086]	0.056 [0.032-0.086]		
Post	0.053 [0.033-0.081]	0.057 [0.027-0.089]	0.051 [0.023-0.071]	-0.008 ± 0.006	-0.011 ± 0.006
Beta-carotene, mg/L					
Pre	0.797 [0.317-1.149]	0.781 [0.443-1.399]	0.737 [0.535-1.144]		
Post	0.787 [0.365-1.040]	0.925 [0.528-1.602]	1.158 [0.630-1.460]	0.438 ± 0.137[6]	0.010 ± 0.144

[1]Results are presented as mean ± SD or median [IQR]. n=30. C, control; LN, low nitrate; HN, high nitrate.

[2]Low nitrate post treatment (n=29).

[3]Between-treatment differences are for the post-treatment values of the high nitrate (HN) compared with low nitrate (LN) and control (C) treatments, adjusted for pre-treatment values, treatment period and treatment order using repeated measures mixed modelling.

[4]P<0.001.

[5]P<0.0001.

[6]P<0.01

Table 4. Descriptive statistics for standard biochemical analyses by treatment, and the between-treatment differences[1]

	C (n=30)	LN (n=30)	HN (n=30)	Treatment effect[2]	
				HN vs. C	HN vs. LN
Plasma total cholesterol, mmol/L					
Pre	5.5 ± 1.0	5.5 ± 1.2	5.5 ± 1.2		
Post	5.6 ± 1.3	5.5 ± 1.2	5.3 ± 1.1	-0.33 ± 0.14	-0.23 ± 0.14
Plasma triglycerides, mmol/L					
Pre	1.0 [0.9-1.5]	1.1 [0.8-1.7]	1.1 [0.8-1.4]		
Post	1.1 [0.9-1.6]	1.2 [0.9-1.8]	1.0 [0.7-1.6]	-0.10 ± 0.07	0.14 ± 0.07
Plasma LDL cholesterol, mmol/L					
Pre	3.4 ± 0.9	3.4 ± 1.0	3.5 ± 1.1		
Post	3.6 ± 1.1	3.5 ± 1.1	3.4 ± 1.0	-0.25 ± 0.12	0.15 ± 0.13
Plasma HDL cholesterol, mmol/L					
Pre	1.5 ± 0.4	1.4 ± 0.4	1.4 ± 0.3		
Post	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	-0.03 ± 0.03	-0.03 ± 0.03
Plasma glucose, mmol/L					
Pre	5.1 ± 0.5	4.9 ± 0.4	5.0 ± 0.6		
Post	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.5	0.01 ± 0.08	-0.01 ± 0.08
Plasma creatinine, mmol/L					
Pre	69.9 ± 10.1	70.5 ± 10.6	70.1 ± 12.1		
Post	70.5 ± 10.0	71.2 ± 11.2	69.2 ± 10.0	-1.35 ± 1.31	-2.14 ± 1.37
Plasma sodium, mmol/L					
Pre	137.0 [134.5-138.7]	137.3 [135.6-138.1]	137.0 [134.9-138.9]		
Post	137.5 [134.9-138.9]	137.0 [135.7-138.1]	136.2 [133.3-138.4]	-0.12 ± 1.2	-0.05 ± 1.29

Plasma potassium, mmol/L					
Pre	4.1 ± 0.3	4.0 ± 0.3	4.0 ± 0.3		
Post	4.0 ± 0.3	4.0 ± 0.3	3.9 ± 0.3	-0.07 ± 0.06	-0.03 ± 0.06
Urinary sodium, mmol/mmol creatinine					
Pre	8.2 [5.0-10.8]	8.3 [5.5-12.7]	8.9 [4.4-11.1]		
Post	7.0 [4.7-11.3]	8.0 [6.6-11.1]	7.1 [5.3-10.5]	0.44 ± 1.05	-0.70 ± 1.10
Urinary potassium, mmol/mmol creatinine					
Pre	7.3 [5.9-8.7]	7.4 [5.9-10.0]	7.8 [5.6-10.1]		
Post	8.1 [7.1-9.3]	7.8 [5.9-9.8]	8.1 [6.5-10.4]	0.22 ± 0.54	0.35 ± 0.56
Urinary sodium/potassium ratio, mmol/mmol creatinine					
Pre	1.0 [0.8-1.5]	1.1 [0.8-1.5]	0.9 [0.6-1.5]		
Post	0.8 [0.6-1.3]	1.0 [0.8-1.5]	0.9 [0.6-1.3]	0.07 ± 0.11	0.12 ± 0.12

[1]Results are presented as mean ± SD or median [IQR]. n=30. C, control; LN, low nitrate; HN, high nitrate.

[2]Between-treatment differences are for the post-treatment values of the high nitrate (HN) compared with low nitrate (LN) and control (C) treatments, adjusted for pre-treatment values, treatment period and treatment order using repeated measures mixed modelling. There were no significant differences between treatments.

Table 5. Descriptive statistics for blood pressure by treatment, and the between-treatment differences[1]

	C (n=30)	LN (n=30)	HN (n=30)	Treatment effect[2]	
				HN vs. C	HN vs. LN
Ambulatory blood pressure[3]					
Overall 24-hour					
SBP mean, mmHg					
Pre	126.5 ± 7.8	126.6 ± 6.4	125.6 ± 6.8		
Post	125.9 ± 7.4	127.9 ± 8.5	126.5 ± 6.1	1.1 ± 1.3	-1.3 ± 1.3
DBP mean, mmHg					
Pre	75.3 ± 8.6	75.3 ± 7.5	75.2 ± 7.8		
Post	75.0 ± 8.3	75.7 ± 8.9	75.2 ± 7.9	0.4 ± 0.9	-0.6 ± 1.0
HR mean, beats/min					
Pre	68.0 ± 8.5	69.3 ± 8.5	68.8 ± 8.3		
Post	68.2 ± 8.3	68.6 ± 8.2	68.6 ± 8.0	-0.2 ± 1.0	0.3 ± 1.0
Day time					
SBP mean, mmHg					
Pre	130.4 ± 8.0	130.7 ± 6.7	129.8 ± 7.0		
Post	130.2 ± 7.9	132.0 ± 9.0	130.6 ± 6.4	0.7 ± 1.5	-1.3 ± 1.5
DBP mean, mmHg					
Pre	78.4 ± 8.8	78.8 ± 7.9	78.5 ± 8.2		
Post	78.3 ± 8.7	78.9 ± 9.4	78.4 ± 8.5	0.1 ± 1.1	-0.5 ± 1.1
HR mean, beats/min					
Pre	70.7 ± 9.3	72.3 ± 9.0	71.5 ± 8.7		
Post	70.6 ± 8.9	71.5 ± 8.8	71.2 ± 8.6	-0.1 ± 1.1	0.3 ± 1.1
Night time					
SBP mean, mmHg					
Pre	114.9 ± 9.4	113.5 ± 7.8	112.5 ± 8.5		
Post	112.6 ± 8.4	115.3 ± 10.4	114.2 ± 8.9	3.0 ± 1.7	-1.1 ± 1.7

DBP mean, mmHg					
Pre	66.0 ± 8.8	64.7 ± 7.4	65.3 ± 8.1		
Post	64.8 ± 8.0	66.0 ± 8.8	65.5 ± 7.9	1.0 ± 1.0	-1.0 ± 1.1
HR mean, beats/min					
Pre	60.0 ± 7.6	60.1 ± 7.6	60.5 ± 8.2		
Post	60.7 ± 7.7	59.7 ± 7.6	60.7 ± 7.3	-0.4 ± 1.1	0.4 ± 1.2
Home blood pressure					
Overall 4-week					
SBP mean, mmHg					
Pre	128.2 ± 10.2	126.9 ± 9.9	129.3 ± 10.1		
Post	128.0 ± 9.5	126.9 ± 10.0	127.8 ± 9.7	-0.9 ± 0.7	-1.3 ± 0.8
DBP mean, mmHg					
Pre	75.1 ± 8.8	74.5 ± 8.6	75.6 ± 9.0		
Post	75.3 ± 8.6	74.5 ± 9.0	74.5 ± 8.9	-0.6 ± 0.6	-1.2 ± 0.6
HR mean, beats/min					
Pre	64.6 ± 8.8	65.2 ± 9.0	65.5 ± 8.9		
Post	65.1 ± 8.6	64.9 ± 8.8	65.7 ± 8.3	-0.2 ± 0.5	0.6 ± 0.6
Clinic blood pressure					
SBP mean, mmHg					
Pre	130.4 ± 8.6	129.8 ± 10.0	130.2 ± 7.8		
Post	130.0 ± 7.6	129.7 ± 8.4	128.4 ± 8.9	-1.4 ± 1.6	-1.9 ± 1.7
DBP mean, mmHg					
Pre	77.4 ± 7.1	75.2 ± 7.6	76.6 ± 7.0		
Post	76.5 ± 5.7	76.2 ± 8.4	75.3 ± 7.2	-0.6 ± 1.0	-2.3 ± 1.1
HR mean, beats/min					
Pre	57.2 ± 7.5	56.3 ± 7.9	57.9 ± 8.0		
Post	56.9 ± 7.3	56.9 ± 7.6	58.3 ± 8.1	-0.5 ± 1.0	-0.2 ± 1.1

[1]Results are presented as mean ± SD. n=30. C, control; LN, low nitrate; HN, high nitrate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

[2]Between-treatment differences are for the post-treatment values of the high nitrate (HN) compared with low nitrate (LN) and control (C) treatments, adjusted for pre-treatment values, treatment period and treatment order using repeated measures mixed modelling. Home blood pressure pre-treatment values that were adjusted for were the last 7 days prior to treatment. There were no significant differences between treatments.

[3]n=28.

Table 6. Descriptive statistics for pulse wave analysis and pulse wave velocity results by treatment, and the between-treatment differences[1]

	C (n=30)	LN (n=30)	HN (n=30)	Treatment effect[2]	
				HN vs. C	HN vs. LN
Central systolic pressure, mmHg					
Pre	119.8 ± 8.7	118.9 ± 10.2	118.8 ± 7.8		
Post	119.1 ± 8.1	118.7 ± 8.6	117.8 ± 9.1	-0.6 ± 1.5	-1.1 ± 1.5
Central diastolic pressure, mmHg					
Pre	77.4 ± 7.2	75.4 ± 7.6	76.9 ± 7.1		
Post	76.5 ± 5.3	76.3 ± 8.3	75.8 ± 7.4	-0.4 ± 1.0	-2.0 ± 1.1
Central augmentation index, %					
Pre	29.5 ± 11.2	27.6 ± 10.5	29.3 ± 11.4		
Post	29.8 ± 10.2	28.5 ± 10.9	28.8 ± 10.0	-0.7 ± 1.3	0.7 ± 1.4
Pulse wave velocity[3], m/s					
Pre	8.2 ± 1.5	8.3 ± 1.1	8.3 ± 1.1		
Post	8.3 ± 1.3	8.3 ± 0.9	8.3 ± 1.1	-0.1 ± 0.1	-0.1 ± 0.1

[1]Results are presented as mean ± SD. n=30. C, control; LN, low nitrate; HN, high nitrate.

[2]Between-treatment differences are for the post-treatment values of the high nitrate (HN) compared with low nitrate (LN) and control (C) treatments, adjusted for pre-treatment values, treatment period and treatment order using repeated measures mixed modelling. There were no significant differences between treatments.

[3]n=26.

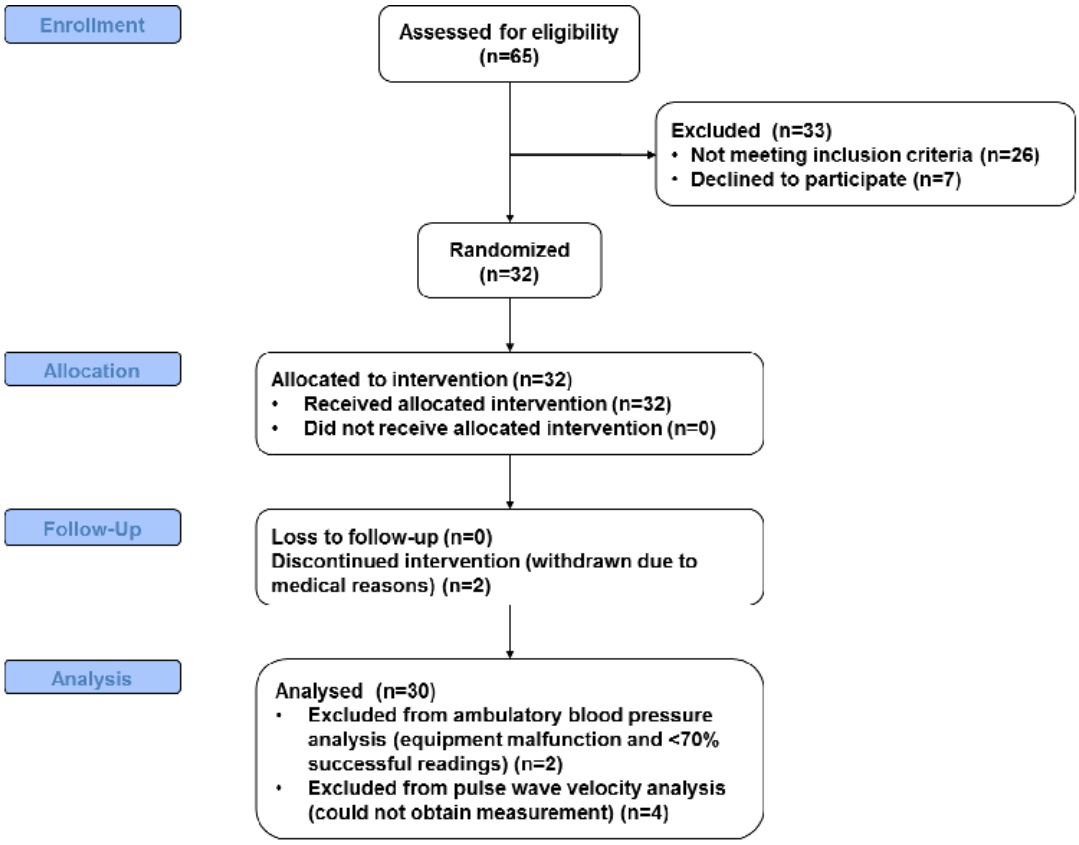

Figure 1. CONSORT flow diagram for participant recruitment.

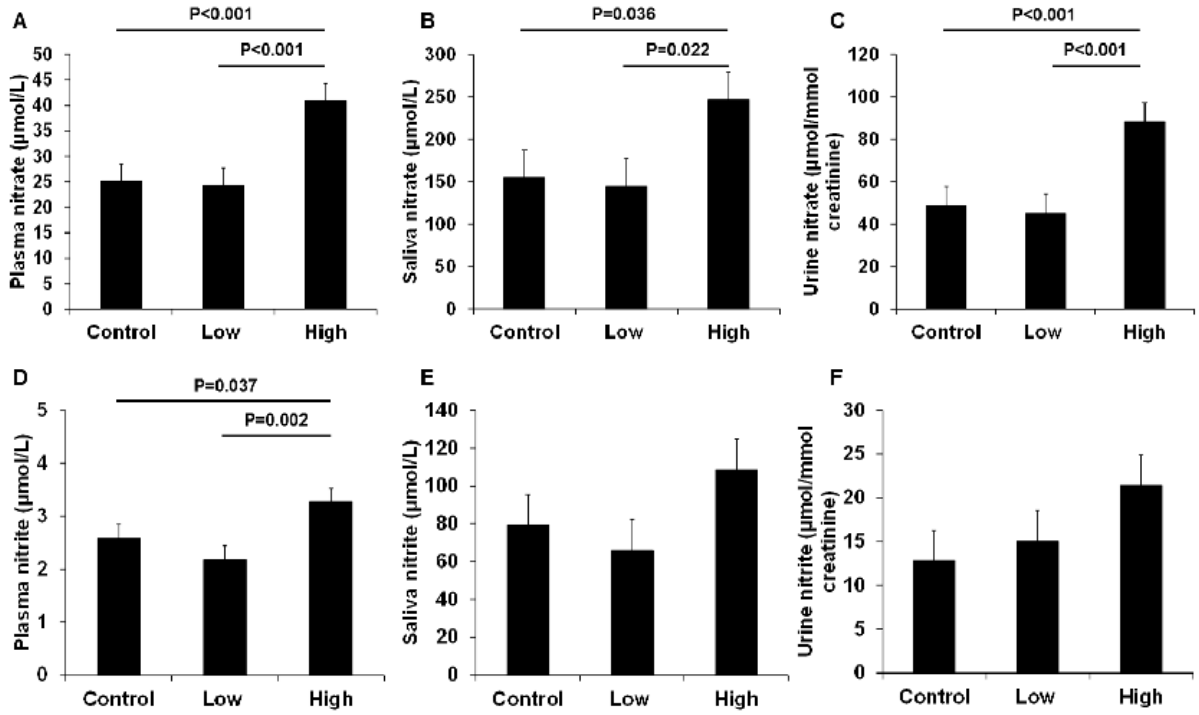

Figure 2. The effects of 4 weeks high and low nitrate vegetable juice on nitrate concentrations in (A) plasma, (B) saliva, and (C) urine; and nitrite concentrations in (D) plasma, (E) saliva, and (F) urine. Results are presented as estimated mean ± SE adjusted for pre-treatment values, treatment period and treatment order using repeated measures mixed modelling (n=30).

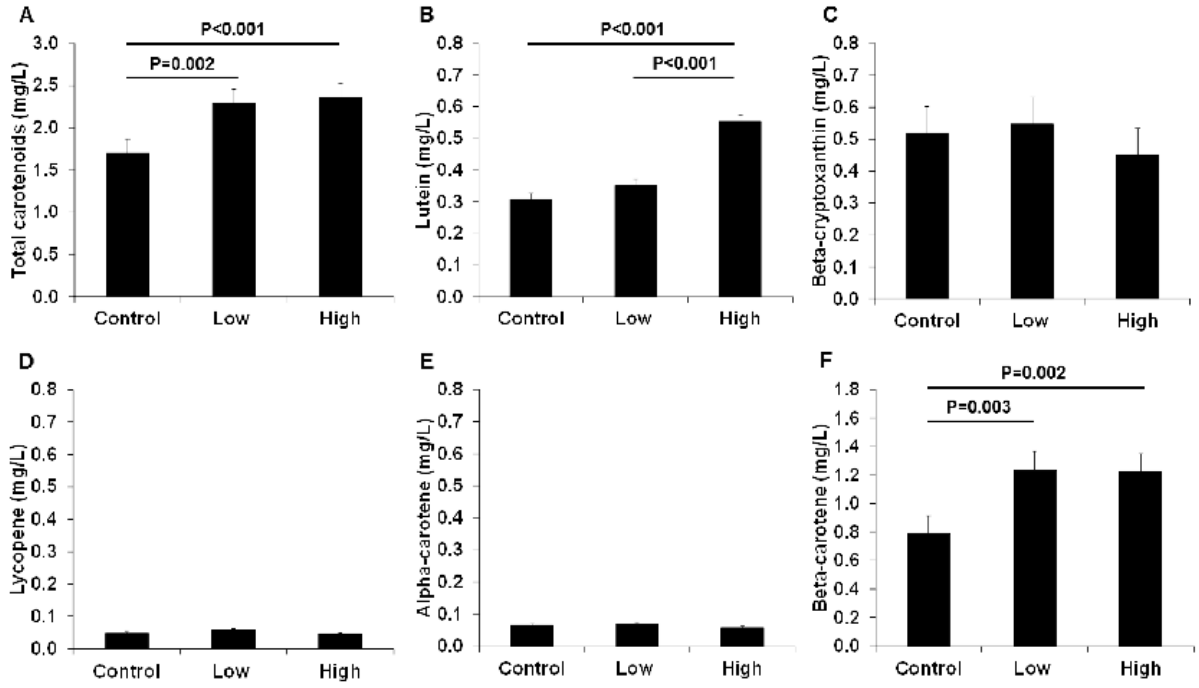

Figure 3. The effects of 4 weeks high and low nitrate vegetable juice on plasma concentrations of (A) total carotenoids, (B) lutein, (C) beta-cryptoxanthin, (D) lycopene, (E) alpha-carotene and (F) beta-carotene. Results are presented as estimated mean ± SE adjusted for pre-treatment values, treatment period and treatment order using repeated measures mixed modelling (n=30). Low nitrate treatment (n=29).

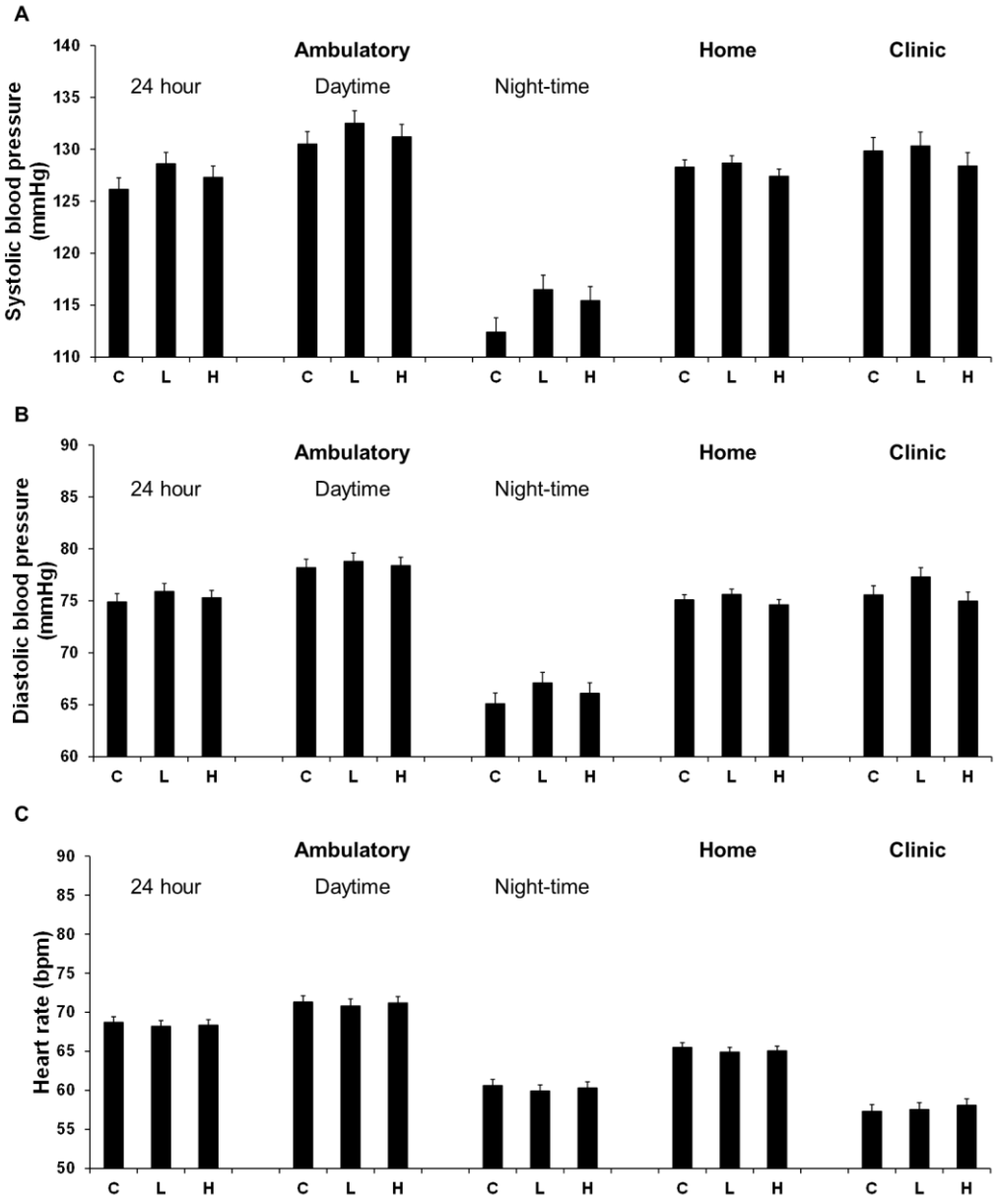

Figure 4. The effects of 4 weeks high and low nitrate vegetable juice on ambulatory, home and clinic measures of (A) systolic blood pressure, (B) diastolic blood pressure, and (C) heart rate. Results are presented as estimated mean ± SE adjusted for pre-treatment values, treatment period and treatment order using repeated measures mixed modelling. There were no significant differences between treatments. Home measures of blood pressure consisted of the last 7 days of treatment adjusted for the 7 days prior to pre-treatment. Ambulatory blood pressure (n=28); home and clinic blood pressure (n=30). C, control; L, low nitrate; H, high nitrate.

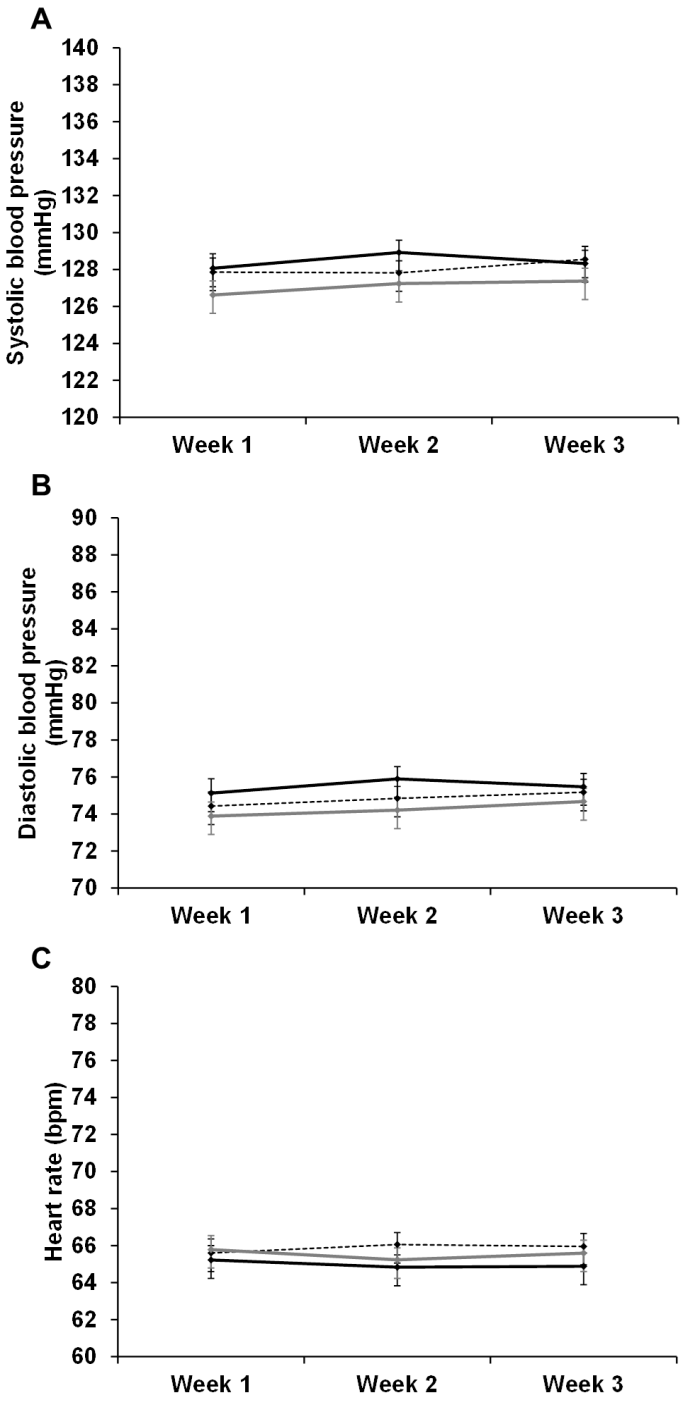

Figure 5. The effects of high and low nitrate vegetable juice on home measures of (A) systolic blood pressure, (B) diastolic blood pressure, and (C) heart rate for week 1, week 2, and week 3. Results are presented as estimated mean ± SE adjusted for the 7 days prior to pre-treatment, treatment period and treatment order using repeated measures mixed modelling (n=30). There were no significant differences between treatments.