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## Nitrate-rich vegetables do not lower blood pressure in individuals with mildly elevated blood pressure: A 4-wk randomized controlled crossover trial

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

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

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

46 **ABSTRACT**

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

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

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

61 **Results:** Plasma nitrate and nitrite concentrations were increased with the HN treatment in  
62 comparison to the LN and control treatments ( $P < 0.001$ ). Plasma carotenoids were increased  
63 with the HN and LN treatments compared to the control ( $P < 0.01$ ). High nitrate treatment did  
64 not reduce systolic blood pressure (24-hour ambulatory: HN  $127.4 \pm 1.1$  mmHg, LN  $128.6 \pm 1.1$   
65 mmHg, C  $126.2 \pm 1.1$  mmHg,  $P = 0.20$ ; home: HN  $127.4 \pm 0.7$  mmHg, LN  $128.7 \pm 0.7$  mmHg, C  
66  $128.3 \pm 0.7$  mmHg,  $P = 0.36$ ; clinic: HN  $128.4 \pm 1.3$  mmHg, LN  $130.3 \pm 1.3$  mmHg, C  $129.8 \pm 1.3$   
67 mmHg,  $P = 0.49$ ) or diastolic blood pressure compared with LN and C treatments ( $P > 0.05$ )  
68 after adjustment for pre-treatment values, treatment period and treatment order. Similarly, no  
69 differences were observed between treatments for arterial stiffness measures ( $P > 0.05$ ).

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

73 **Clinical trial registry number and website:** This trial was prospectively registered at  
74 [www.anzctr.org.au](http://www.anzctr.org.au) as ACTRN12615000194561.

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

## 76 INTRODUCTION

77 High blood pressure is the leading risk factor for global disease burden (1). The maintenance  
78 of a healthy blood pressure and the prevention of hypertension continues to be a public health  
79 priority worldwide (2, 3). It is estimated that for every 10 mmHg reduction in systolic blood  
80 pressure there is a 13-28% lower risk of cardiovascular disease events and all-cause mortality  
81 (4). Arterial stiffness, which is closely related to hypertension (5, 6), is also a predictor of  
82 cardiovascular and all-cause mortality (7). Diets rich in vegetables, such as the vegetarian diet  
83 (8), Mediterranean diet, Nordic diet, and Dietary Approaches to Stop Hypertension (DASH)  
84 diet (9), have been shown to lower blood pressure. There are many components of a diet rich  
85 in vegetables that may benefit cardiovascular health. In particular, emerging evidence  
86 suggests that inorganic nitrate found in vegetables can contribute to lowering blood pressure  
87 (10, 11).

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

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

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

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



## 110 **METHODS**

### 111 **Ethics**

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

### 116 **Trial design**

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

### 127 **Participants**

128 Thirty participants with pre-hypertension or untreated grade 1 hypertension were recruited  
129 from the Perth general population using newspaper advertisements between March and July  
130 2015. Participants were ambulant men and women between the ages of 21 and 75 years, with  
131 a resting mean systolic blood pressure between 120-160 mmHg, inclusive. Participants were  
132 excluded if they were: diabetic; a smoker; taking antihypertensive medication, nitric oxide  
133 donors, organic nitrates and nitrites, or related drugs; had a body mass index (BMI)  $\geq 35$  kg/m<sup>2</sup>  
134 or  $< 18.5$  kg/m<sup>2</sup>; or used antibacterial mouth wash. For a complete list of inclusion and

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

### 151 **Dietary interventions**

152 During each 4-week treatment period, additions were made to participants' breakfast and  
153 dinner, consisting of vegetables blended into juices or a matching control juice. Participants  
154 were asked to maintain all meals as usual with the exception of limiting high nitrate  
155 vegetables where possible. In the high and low nitrate treatment periods participants were  
156 asked to consume 100 g/d of high and low nitrate vegetables, respectively, before breakfast  
157 and dinner. This was equivalent to increasing vegetable intake by approximately 2.7 serves/d  
158 (~200-300 kJ/d) (24). For the control treatment, participants were asked to blend a quarter of  
159 an orange and 8 g maltodextrin in water (approximately energy matched to the vegetables

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

#### 174 **Clinical measurements**

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

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

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

## 188 **Blood pressure**

### 189 *Ambulatory blood pressure*

190 Ambulatory blood pressure and heart rate were monitored every 20 minutes during the day  
191 and every 30 minutes overnight for a 24-hour period commencing at the end of pre- and post-  
192 treatment visits, as previously described (26). Measurements showing an error code or those  
193 with a pulse pressure of less than 20 mmHg were excluded from the analysis. Blood pressure  
194 traces that were missing more than four hourly means over the 24 hours were also excluded  
195 from the analysis. A minimum of 70% successful readings was considered a valid recording.  
196 Mean blood pressure was determined for the 24-hour, daytime (06:00-21:59), and night-time  
197 (22:00-05:59) period.

### 198 *Home blood pressure*

199 Participants were provided with a digital blood pressure monitor (UA-767PC, A&D Co., Ltd,  
200 Saitama, Japan) and an appropriately sized upper-arm cuff. Participants were given  
201 appropriate training and instructions on its use prior to the study commencing. Home blood  
202 pressure was measured and recorded by each participant three times daily (shortly after  
203 waking and prior to breakfast, 1-2 hours prior to dinner, and 1-2 hours after dinner) for the  
204 entire study duration. Participants were instructed to rest for 5 minutes prior to commencing  
205 three blood pressure readings over a 3-minute period. The first measurement was excluded  
206 from the analysis and the mean of the second and third measurements used. Mean blood  
207 pressure was determined for the overall 4-week pre- and post-treatment periods; week 1, week  
208 2, and week 3 of each treatment; the last 7 days pre- and post-treatment period; and the  
209 morning, afternoon, and evening of the last 7 days pre- and post-treatment period.

### 210 *Clinic blood pressure*

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

### 218 **Arterial stiffness**

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

### 232 **Biochemical analyses**

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

235 Gel (LH PST II) tubes. Whole blood was centrifuged at 4<sup>0</sup>C, 3500 rpm for 10 minutes and  
236 plasma aliquots stored at -80<sup>0</sup>C until analysis. Participants spat into sterile polystyrene jars for  
237 5 minutes to obtain saliva samples and saliva aliquots stored at -80<sup>0</sup>C until analysis. Spot  
238 urine samples were also collected into sterile polystyrene jars and aliquots stored at -80<sup>0</sup>C  
239 until analysis.

#### 240 *Nitrate and nitrite analysis*

241 Nitrate and nitrite concentrations were measured in plasma, saliva, urine, and juice samples  
242 using gas chromatography/mass spectrometry (GCMS) with <sup>15</sup>N-labelled nitrate and nitrite as  
243 internal standards, as previously described (27).

#### 244 *Plasma carotenoid analysis*

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

#### 254 *Other biochemical analyses*

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

#### 259 **Statistics**

260 *Sample size*

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

267 *Statistical methods*

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

279 **RESULTS**

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

284 Baseline demographic and clinical characteristics for the 30 participants are shown in **Table**  
285 **1**. Baseline dietary data is shown in **Supplemental Table 4**. At baseline, the mean (SD) total  
286 nitrate, estimated using a nitrate content of vegetables database (31) as well as published food  
287 composition data, was 84.7 (36.2) mg/d (81.9% from vegetables alone). The mean (SD) total  
288 vegetable intake was 181.8 (75.8) g/d. Using participants' food diaries from each treatment  
289 period, the median (IQR) reported increase in nitrate-rich vegetables for the high nitrate  
290 treatment was 201.5 (198.7-204.1) g/d and the increase in nitrate-poor vegetables for the low  
291 nitrate treatment was 201.7 (197.0-207.7) g/d. Descriptive statistics for the individual nitrate-  
292 rich and nitrate-poor vegetables consumed in both the high and low nitrate treatments are  
293 shown in **Supplemental Table 3**. The median (IQR) nitrate and nitrite concentrations, from  
294 the juice samples provided by participants, and in which the participants were estimated to be  
295 consuming, were: 149.1 (118.2-237.0) and 3.96 (2.19-5.20) mg/d for the high nitrate  
296 treatment (n=30); 21.5 (13.9-29.9) and 0.31 (0.16-0.48) mg/d for the low nitrate treatment  
297 (n=27); and 5.5 (3.7-8.0) and 0.06 (0.05-0.08) mg/d for the control (n=29), respectively.  
298 Nitrate and nitrite concentrations were significantly different between treatment juices  
299 ( $P < 0.001$  for both). High nitrate juices were 27-fold higher in nitrate and 66-fold higher in  
300 nitrite concentration than that of the control ( $P < 0.001$  for both). In addition, the high nitrate  
301 juices were 6.9-fold higher in nitrate and 12.8-fold higher in nitrite concentration than that of  
302 the low nitrate juices ( $P < 0.001$  for both). The low nitrate and control juices were not different  
303 in nitrate ( $P = 0.326$ ) and nitrite ( $P = 0.779$ ) concentrations.



## 304 **Compliance**

305 Compliance was measured for the three treatments using self-reported food diaries.  
306 Participants recorded the weight (g) of each vegetable consumed in their juice on a daily  
307 basis. Compliance was calculated by dividing the number of days the vegetables were  
308 consumed by the number of days the vegetables should have been consumed and then  
309 multiplying by 100. On the basis of reported consumption of treatments using food diaries,  
310 the median (IQR) compliance was 98.1 (90.6-100.0%) for the high nitrate treatment, 98.1%  
311 (92.9-100.0%) for the low nitrate treatment, and 98.1% (96.0-100.0%) for the control  
312 treatment. Compliance for the control diet was also measured by dividing the amount (g) of  
313 returned maltodextrin by the amount (g) of maltodextrin administered to participants before  
314 their treatment period and then multiplying by 100. On the basis of returned maltodextrin, the  
315 median (IQR) compliance was 131.5% (110.7-142.5%). All treatment diets were well  
316 tolerated. Whilst on the control treatment, one participant reported constipation symptoms and  
317 another reported loose stools. No serious adverse events were reported.

## 318 **Biomarkers of intake and metabolism**

### 319 *Plasma nitrate and nitrite*

320 Median (IQR) plasma nitrate and nitrite concentrations pre- and post-treatment are shown in  
321 **Table 2**. Plasma nitrate was significantly different between treatments ( $P < 0.001$ ) (**Figure 2**).  
322 There was a 1.7-fold increase in plasma nitrate concentrations for the high nitrate treatment  
323 compared to the low nitrate treatment ( $P < 0.001$ ) and a 1.6-fold increase for the high nitrate  
324 treatment compared to control ( $P < 0.001$ ) (Figure 2). Plasma nitrite was significantly different  
325 between treatments ( $P = 0.007$ ) (Figure 2). There was a 1.5-fold increase in plasma nitrite  
326 concentrations for the high nitrate treatment compared to the low nitrate treatment ( $P = 0.002$ )  
327 and a 1.3-fold increase for the high nitrate treatment compared to control ( $P = 0.037$ ) (Figure  
328 2).

329 *Salivary nitrate and nitrite*

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

336 *Urinary nitrate and nitrite*

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

343 *Plasma carotenoids*

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

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

#### 357 *Plasma and urinary sodium and potassium*

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

### 360 **Blood pressure**

#### 361 *Ambulatory blood pressure*

362 Ambulatory measures of blood pressure were excluded for 2 (6.7%) participants due to  
363 equipment malfunction (n=1) and <70% successful readings (n=1). The mean (SD) 24-hour,  
364 daytime, and night-time ambulatory measures of blood pressure and heart rate pre- and post-  
365 treatment are presented in **Table 5**. There were no significant differences between treatments  
366 for mean 24-hour, daytime, or night-time ambulatory blood pressure and heart rate (**Figure**  
367 **4**). No carryover effects were observed for 24-hour, daytime, and night-time ambulatory  
368 measures of blood pressure and heart rate (P>0.05 for all). In a post-hoc sensitivity analysis in  
369 which we adjusted for age, gender and BMI, the results were very similar and not  
370 substantively changed (data not shown).

#### 371 *Home blood pressure*

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

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

### 382 *Clinic blood pressure*

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

### 387 **Arterial stiffness**

388 Pulse wave analysis was complete for all participants. Pulse wave velocity was incomplete for  
389 4 (13.3%) participants due to an inability to obtain measurements. Mean (SD) central systolic  
390 and diastolic pressures, central augmentation index (%), and pulse wave velocity for pre- and  
391 post-treatment are shown in **Table 6**. No significant differences were observed between  
392 treatments for central systolic blood pressure, central diastolic blood pressure, central  
393 augmentation index, and pulse wave velocity.

### 394 **Plasma lipids and glucose**

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

### 397 **Anthropometry, physical activity and alcohol intake**

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

**403 DISCUSSION**

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

413 A number of short-term trials have assessed the effects of increased nitrate intake on blood  
414 pressure (10, 11). The results of these studies are inconsistent, with several trials finding  
415 lower blood pressure with increased nitrate intake (19, 32-37), and others finding no effect  
416 (20, 38-42). Our study is unique in that the design assessed the impact of a sustained 4-week  
417 increase in nitrate-rich vegetables in individuals with pre-hypertension or untreated grade 1  
418 hypertension and that subjects were not taking anti-hypertensive medication which could have  
419 modified any effect of nitrate. There are many potential factors that could influence whether  
420 an increase in nitrate intake results in lower blood pressure. The duration of the study appears  
421 to be a factor, with acute studies consistently showing blood pressure lowering effects (17,  
422 42-53). Other factors may include the dose of nitrate provided; the background nitrate and  
423 vegetable intake of the study participants, which may alter the effective dose; whether there is  
424 an individual threshold level, beyond which there is little additional benefit; and the age and  
425 health status of the participants.

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

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

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

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

457 Limitations to this intervention study need to be considered. Firstly, we did not observe  
458 differences in urinary potassium concentrations. We may have expected to see a >10%  
459 increase in potassium excretion given the self-reported increases in vegetable intake. This  
460 could be the result of using creatinine-adjusted values from a spot urine instead of a 24-hour  
461 urine sample to assess urinary potassium (57). We did, however, observe a ~1.6 fold increase  
462 in plasma nitrate and nitrite with increased nitrate-rich vegetables and a ~1.4 fold increase in  
463 plasma total carotenoids with increased nitrate-rich and nitrate-poor vegetables. The increase  
464 in plasma nitrate, nitrite, and total carotenoids gives us confidence the participants were  
465 compliant. It should be noted that plasma nitrite concentrations in our study are higher than  
466 some reported values in the literature. This could be due to a number of factors; however, it is  
467 likely an outcome of the GCMS method compared to gas phase chemiluminescence (27).  
468 Secondly, our study demonstrated no statistical difference in salivary and urinary nitrite  
469 concentrations. Circulating nitrate and nitrite concentrations depend on when nitrate is last  
470 ingested. The half-life of nitrate in plasma is 5-6 hours (58). Nitrite is likely to be similar as  
471 new nitrite is being continuously generated from the ingested nitrate. As participants were  
472 asked to fast for at least 12 hours prior to providing samples, this may explain why no  
473 increases in salivary and urinary nitrite concentrations were observed. However, other  
474 mechanisms may explain this observation and warrants further investigation. Thirdly, the  
475 estimated nitrate intake from the measured juice samples was 149 mg/d, whereas the  
476 estimated nitrate intake using a comprehensive international nitrate database (31) was 326  
477 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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499 designed research; LCB, NPB, CPB, LGW, IBP, and NCW conducted research; LCB, RJW,  
500 and JMH analysed data; LCB and JMH wrote paper; LCB and JMH had primary  
501 responsibility for final content; all authors critically revised the manuscript for important  
502 intellectual content. All authors read and approved the final manuscript.

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**Table 1.** Demographic and clinical characteristics of study participants at screening<sup>1</sup>

	<b>All participants n = 30</b>
<b>Demographics</b>	
Male/female, n	20/10
Age, years	63.0 [55.5-70.5]
BMI, kg/m <sup>2</sup>	27.0 ± 3.9
Waist circumference <sup>2</sup> , cm	89.5 ± 11.7
Hip circumference <sup>2</sup> , cm	102.0 [95.0-104.5]
Waist-to-hip ratio <sup>2</sup>	0.9 ± 0.1
Smoking history <sup>2</sup>	11 (37.9)
<b>Medications</b>	
HMG-CoA reductase inhibitors	5 (16.7)
<b>Clinic blood pressure</b>	
Systolic blood pressure, mmHg	133.6 ± 8.4
Diastolic blood pressure, mmHg	77.7 ± 8.0
Heart rate, bpm	61.6 ± 8.0
<b>Biochemistry</b>	
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

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

<sup>2</sup>n=29.

Table 2. Descriptive statistics for nitrate and nitrite concentrations in plasma, saliva and urine by treatment, and the between-treatment differences<sup>1</sup>

	C (n=30)	LN (n=30)	HN (n=30)	Treatment effect <sup>2</sup>	
				HN vs. C	HN vs. LN
<b>Nitrate</b>					
<b>Plasma, µmol/L</b>					
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 <sup>3</sup>	16.7 ± 4.3 <sup>3</sup>
<b>Salivary, µmol/L</b>					
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 <sup>4</sup>	102.4 ± 43.3 <sup>4</sup>
<b>Urinary, µmol/mmol creatinine</b>					
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 <sup>3</sup>	43.0 ± 11.2 <sup>3</sup>
<b>Nitrite</b>					
<b>Plasma, µmol/L</b>					
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 <sup>4</sup>	1.1 ± 0.3 <sup>4</sup>
<b>Salivary, µmol/L</b>					
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 <sup>4</sup>
<b>Urinary, µmol/mmol creatinine</b>					
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 <sup>4</sup>	6.4 ± 4.0

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

<sup>2</sup>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.

<sup>3</sup>P<0.001.

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<sup>4</sup>P<0.05.

**Table 3.** Descriptive statistics for plasma carotenoid concentrations by treatment, and the between-treatment differences<sup>1</sup>

	C (n=30)	LN (n=30) <sup>2</sup>	HN (n=30)	Treatment effect <sup>3</sup>	
				HN vs. C	HN vs. LN
<b>Total carotenoids, mg/L</b>					
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 <sup>4</sup>	0.073 ± 0.178
<b>Lutein, mg/L</b>					
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 <sup>5</sup>	0.202 ± 0.029 <sup>5</sup>
<b>Beta-cryptoxanthin, mg/L</b>					
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
<b>Lycopene, mg/L</b>					
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
<b>Alpha-carotene, mg/L</b>					
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
<b>Beta-carotene, mg/L</b>					
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 <sup>6</sup>	0.010 ± 0.144

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

<sup>2</sup>Low nitrate post treatment (n=29).

<sup>3</sup>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.

<sup>4</sup>P<0.001.

<sup>5</sup>P<0.0001.

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<sup>6</sup>P<0.01

**Table 4.** Descriptive statistics for standard biochemical analyses by treatment, and the between-treatment differences<sup>1</sup>

	C (n=30)	LN (n=30)	HN (n=30)	Treatment effect <sup>2</sup>	
				HN vs. C	HN vs. LN
<b>Plasma total cholesterol, mmol/L</b>					
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
<b>Plasma triglycerides, mmol/L</b>					
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
<b>Plasma LDL cholesterol, mmol/L</b>					
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
<b>Plasma HDL cholesterol, mmol/L</b>					
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
<b>Plasma glucose, mmol/L</b>					
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
<b>Plasma creatinine, mmol/L</b>					
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
<b>Plasma sodium, mmol/L</b>					
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

<b>Plasma potassium, mmol/L</b>						
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	
<b>Urinary sodium, mmol/mmol creatinine</b>						
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	
<b>Urinary potassium, mmol/mmol creatinine</b>						
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	
<b>Urinary sodium/potassium ratio, mmol/mmol creatinine</b>						
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	

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

<sup>2</sup>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<sup>1</sup>

	C (n=30)	LN (n=30)	HN (n=30)	Treatment effect <sup>2</sup>	
				HN vs. C	HN vs. LN
<b>Ambulatory blood pressure<sup>3</sup></b>					
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	
<b>Home blood pressure</b>						
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	
<b>Clinic blood pressure</b>						
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	

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<sup>1</sup>Results are presented as mean  $\pm$  SD. n=30. C, control; LN, low nitrate; HN, high nitrate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

<sup>2</sup>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.

<sup>3</sup>n=28.

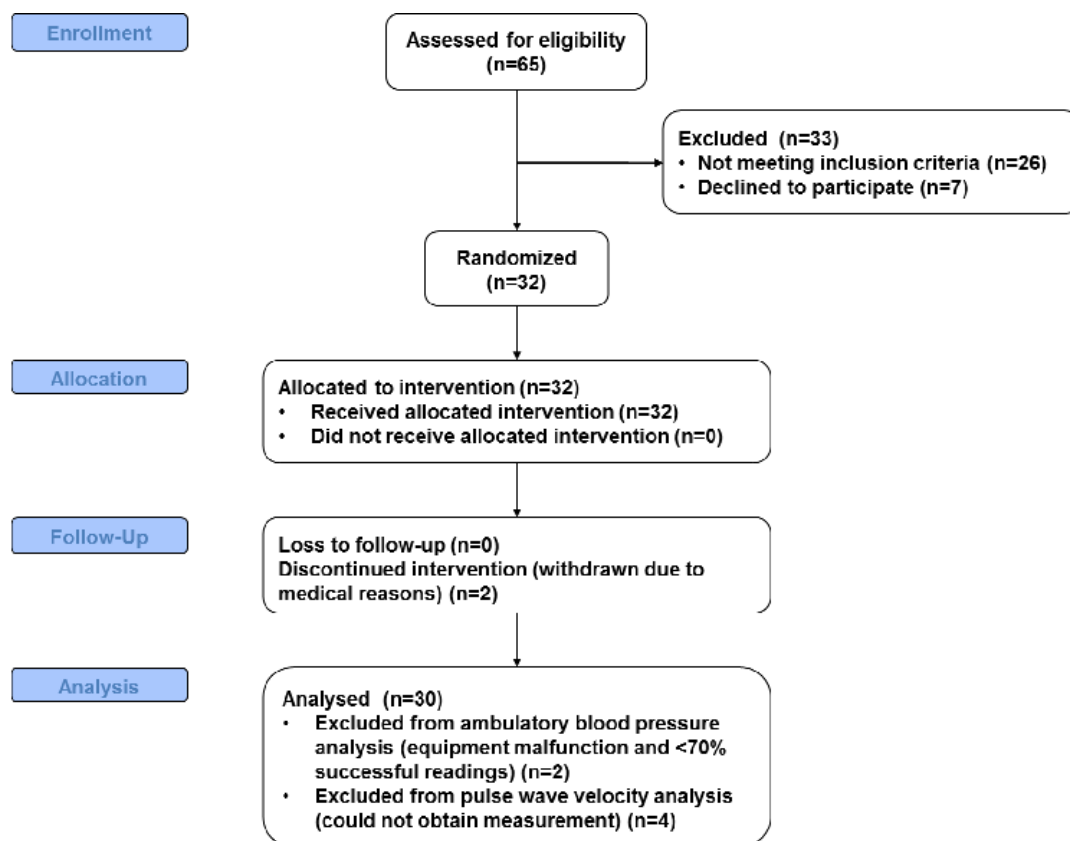
**Table 6.** Descriptive statistics for pulse wave analysis and pulse wave velocity results by treatment, and the between-treatment differences<sup>1</sup>

	C (n=30)	LN (n=30)	HN (n=30)	Treatment effect <sup>2</sup>	
				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 <sup>3</sup> , 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

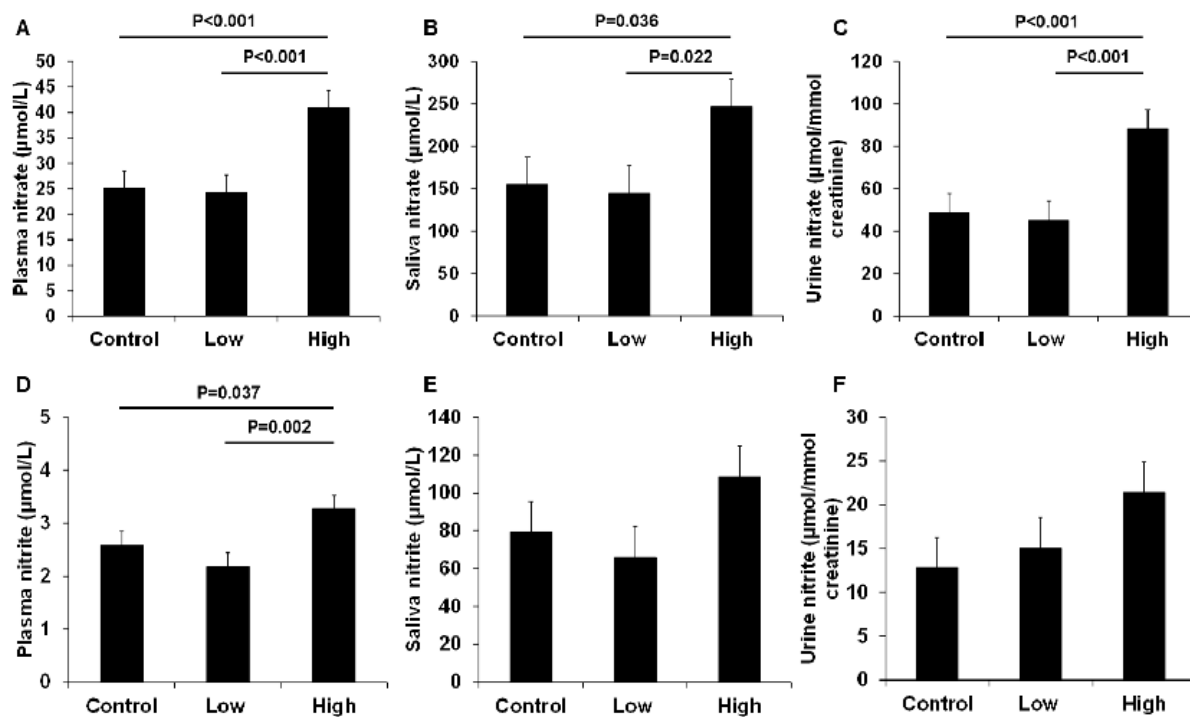
<sup>1</sup>Results are presented as mean ± SD. n=30. C, control; LN, low nitrate; HN, high nitrate.

<sup>2</sup>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.

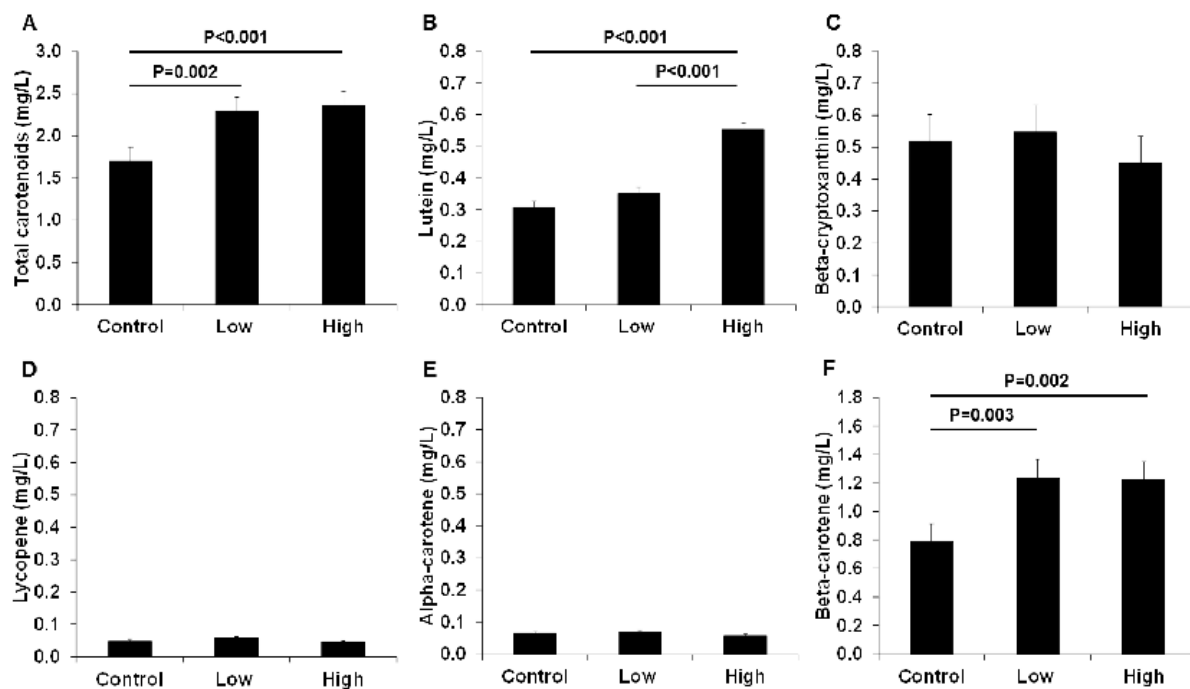
<sup>3</sup>n=26.



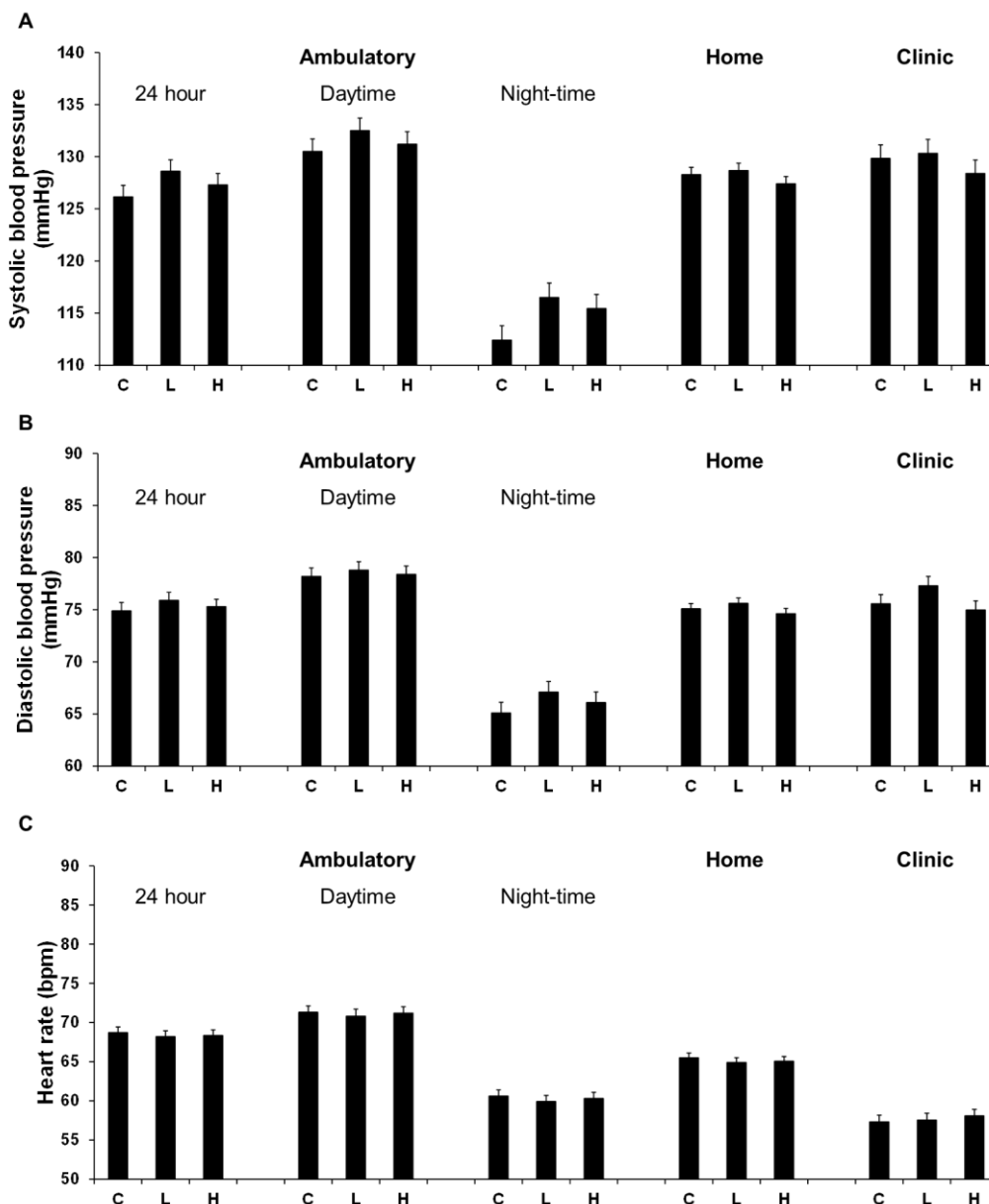
**Figure 1.** CONSORT flow diagram for participant recruitment.



**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  $\pm$  SE adjusted for pre-treatment values, treatment period and treatment order using repeated measures mixed modelling (n=30).

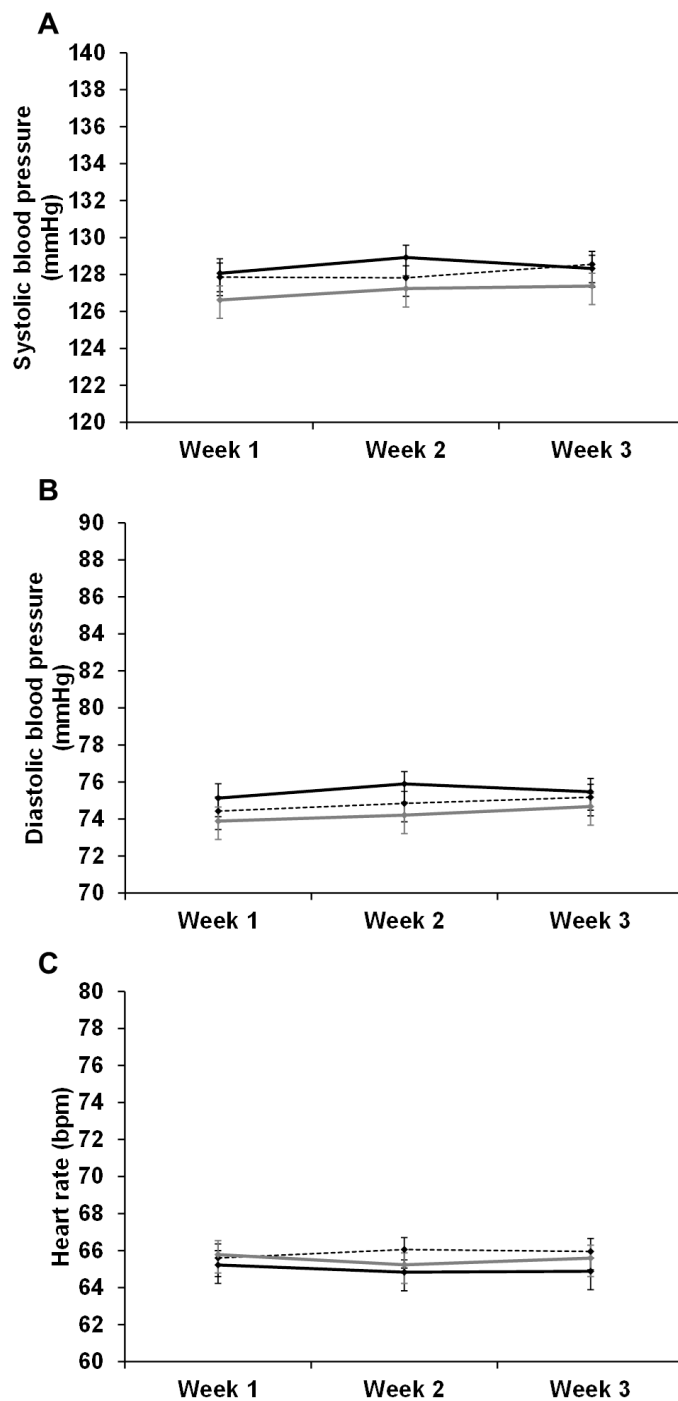


**Figure 3.** The effects of 4 weeks high and low nitrate vegetable juice on plasma concentrations of (A) total carotenoids, (B) lutein, (C) beta-cryptoxanthin, (D) lycopene, (E) alpha-carotene and (F) beta-carotene. Results are presented as estimated mean  $\pm$  SE adjusted for pre-treatment values, 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  $\pm$  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  $\pm$  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.