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Absence of an effect of high nitrate intake from beetroot juice on blood pressure in treated hypertensive individuals: a randomized controlled trial[1]

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ABSTRACT

Background: Dietary nitrate, which is in green leafy vegetables and beetroot, decreases blood pressure through the enterosalivary nitrate-nitrite-nitric oxide pathway in healthy individuals. Whether similar effects would occur in individuals with treated hypertension and, therefore, at increased risk of cardiovascular disease is unclear.

Objective: We assessed whether increased dietary nitrate intake by using beetroot juice for 1 wk lowers blood pressure in treated hypertensive men and women.

Design: Participants (n = 27) were recruited to a randomized, placebo-controlled, double-blind crossover trial. The effect of 1-wk intake of nitrate-rich beetroot juice was compared with 1-wk intake of nitrate-depleted beetroot juice (placebo). The primary outcome was blood pressure assessed by measuring home blood pressure during the intervention and 24-h ambulatory blood pressure on day 7 of the intervention. Other outcomes included nitrate metabolism assessed by measuring nitrate and nitrite in plasma, saliva, and urine.

Results: Relative to the placebo, 1-wk intake of nitrate-rich beetroot juice resulted in a 3-fold increase in plasma nitrite and nitrate, a 7-fold increase in salivary nitrite, an 8-fold higher salivary nitrate, and a 4-fold increase in both urinary nitrite and nitrate ($P < 0.001$). However, no differences in home blood pressure and 24-h ambulatory blood pressure were observed with 1-wk intake of nitrate-rich beetroot juice in comparison with the placebo.

Conclusion: An increase in dietary nitrate intake may not be an effective short-term approach to further lower blood pressure in treated hypertensive subjects. This trial was registered at anzctr.org.au as ACTRN 12613000116729. *Am J Clin Nutr* doi: 10.3945/ajcn.114.101188.

Keywords beetroot, blood pressure, nitrate, nitric oxide, hypertension

INTRODUCTION

Dietary nitrate, through the enterosalivary nitrate-nitrite-nitric oxide (NO) pathway, decreases blood pressure in healthy individuals. To date, >25 clinical trials with healthy participants investigated the effect on blood pressure of increased acute and chronic nitrate intake (1). Reduced blood pressure has been a consistent finding (2). These results suggest that a public health message to increase dietary nitrate intake, which is in high concentrations in green leafy vegetables and beetroot (*Beta vulgaris*), could be an important approach in cardiovascular disease prevention in healthy individuals. However, available data in cardiovascular disease risk groups are limited and not consistent (3–8). It is vital to determine whether increased dietary nitrate intake can reduce blood pressure in individuals with hypertension. This outcome is relevant to population health advice specific to intake of nitrate-rich vegetables in hypertensive individuals.

Dietary nitrate is sequentially reduced through the enterosalivary nitrate-nitrite-NO pathway into physiologically relevant storage pools of NO (9). In the vasculature, NO plays a key role in vascular homeostasis and integrity and, therefore, blood pressure (10). Indeed, high blood pressure is associated with decreased NO production (11). A single dose of dietary nitrate was shown to reduce blood pressure in 15 drug-naive hypertensive men and women 2 h after intake (4). Chronic nitrate intake in older adults with mild hypertension and treated hypertension resulted in decreased systolic blood pressure (5, 6, 8). However, we recently showed that a short-term increase in nitrate derived from green leafy vegetables did not result in lower blood pressure in a group with high-normal range blood pressure (3). In addition, a short-term increase in nitrate intake did not alter blood pressure in a group with type 2 diabetes (7). The effect of a sustained increase in dietary nitrate intake on blood pressure in individuals at increased risk of cardiovascular disease is inconclusive.

The objective of this study was to assess whether increased dietary nitrate intake, by using beetroot juice for 1 wk, lowers

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blood pressure and increases the circulating pool of NO in treated hypertensive men and women. Nitrate metabolism through the enterosalivary nitrate-nitrite-NO pathway was determined by measuring plasma, salivary, and urinary nitrate and nitrite. Blood pressure was assessed by using home and 24-h ambulatory blood pressure monitoring.

METHODS

Participants

Twenty-seven men and women who were taking between 1 and 3 antihypertensive medications for raised blood pressure were recruited by using a newspaper advertisement from the Perth general population. Before enrollment, participants were screened within the University of Western Australia, School of Medicine and Pharmacology, located in the Medical Research Foundation Building at Royal Perth Hospital. After a standard medical history questionnaire, screening comprised electrocardiography, height, weight, BMI, blood pressure measurement, and a routine laboratory analysis of a fasting blood sample. Exclusion criteria were as follows: current or recent smoking (<6 mo); BMI (in kg/m^2) <18 or >35; age <50 or >70 y; systolic blood pressure <120 or >160 mm Hg or diastolic blood pressure >100 mm Hg; use of >3 antihypertensive medications; a change in antihypertensive medication within the previous month; use of antibiotics within the previous month; history of cardiovascular or peripheral vascular disease; diagnosed type 1 or 2 diabetes; history of any major illness such as cancer; a psychiatric illness; current or recent (within previous 6 mo) significant weight loss or gain (>6% of body weight); alcohol consumption >210 g/wk for women and >280 g/wk for men; and women who were pregnant, lactating, or wishing to become pregnant during the study. Participants were requested to avoid the use of mouthwash from 2 wk before their first study visit until completion of the study. The study was carried out in accordance with the Declaration of Helsinki of 1975 as revised in 1983 and was approved by the University of Western Australia Human Research Ethics Committee. Participants provided written informed consent before inclusion in the study. This trial was registered at anzctr.org.au as ACTRN 12613000116729.

Study design

A randomized, placebo-controlled, double-blind crossover trial was performed. Participants were allocated to an intervention plan via block random assignment by using computer-generated random numbers devised by the statistician (RJW). The study ran over a total of 5 wk. During the entire 5 wk, participants consumed a low-nitrate diet. Physical activity, alcohol intake, other lifestyle factors, and medication were not altered during the study. After a 1-wk lead-in period, the study consisted of two 1-wk intervention periods separated by a 2-wk washout period.

Intervention periods comprised a high-nitrate (nitrate-rich beetroot juice; active) intervention and a low-nitrate (nitrate-depleted beetroot juice; placebo) intervention on a background of the low-nitrate diet and unaltered lifestyle. The high-nitrate intervention involved consumption of 2 × 70 mL concentrated nitrate-rich beetroot-juice beverage (70 mL with breakfast and 70 mL with dinner) (Beet it; James White Drinks Ltd.). The low-nitrate diet intervention period involved consumption of 2 × 70 mL nitrate-depleted beetroot juice beverage (70 mL with breakfast and 70 mL with dinner) (Beet it; James White Drinks Ltd.). Active and placebo beetroot juices were identical in appearance and taste with one labeled A and the other labeled B. The lead investigator (JMH) received the randomization codes from the statistician and was responsible for labeling all 70-mL beetroot-juice bottles. Apart from the lead investigator, who did not have any contact with the study participants, all other investigators and research staff were blinded to the treatment allocation.

For the low-nitrate background diet, participants were instructed to avoid intake of vegetables rich in nitrate (including lettuce, beetroot, celery, spinach, Chinese greens, other leafy greens, parsley, and related herbs). A list of foods to avoid or limit was provided to each participant. Because the average dietary nitrate intake in Western populations is estimated to be ~100 mg/d (12), and green leafy vegetable intake is usually not high, the change in diet for most participants was minimal.

Each participant completed a total of 4 visits to the School of Medicine and Pharmacology. These visits were scheduled at day 0 (start of intervention) and day 7 (end of the intervention) for each intervention period. All visits were at the same time of day. Adherence to the study protocol was verified at each visit by using a standard questionnaire. On day 0, participants collected the allocated nitrate-rich or nitrate-depleted beetroot juice, home blood pressure monitor, and a 24-h urine collection bottle. Participants monitored their home blood pressure in the evenings of each intervention period. On day 7, participants arrived at the study unit fasting. They returned a 24-h urine sample collected on day 6 of the intervention. The urine was weighed and stored at −80°C for analysis of urinary nitrate, nitrite, sodium, potassium, and creatinine concentrations. A fasting plasma sample was taken for the analysis of plasma nitrate and nitrite. Thirty minutes after a low-nitrate breakfast (13) with a 70-mL dose of the allocated nitrate-rich or nitrate-depleted beetroot juice, an ambulatory blood pressure monitor was fitted. A saliva sample was taken 120 min after breakfast for the analysis of saliva nitrate and nitrite. Participants consumed a 70-mL dose of the allocated nitrate-rich or nitrate-depleted beetroot juice that evening and the following morning before returning to the study unit to return the ambulatory blood pressure monitor.

Nitrate and nitrite analyses

Concentrations of nitrate and nitrite were measured in each batch of the nitrate-rich and nitrate-depleted beetroot juices used as well as plasma, saliva, and urine samples. Blood samples were collected into tubes containing EDTA, which were immediately centrifuged at 3000 × *g* (15 min at 4°C), and plasma was stored at −80°C until measurement. A 5-min saliva sample was collected and stored at −80°C until measurement. Nitrate and nitrite concentrations in beetroot juices, plasma, saliva, and urine samples were measured by using gas chromatography–mass spectrometry (GC-MS) (14). To determine nitrite and nitrate contaminations from sampling procedures, deionized water was added to tubes containing EDTA and analyzed as previously stated. Measured nitrite and nitrate contaminations were 0.17 and 1.40 μmol/L, respectively. Plasma nitrite and nitrate measures were adjusted accordingly. The GC-MS method for the measurement of plasma nitrite tends to be higher than for the plasma nitrite measured by

using other methods (15). However, for the purpose of establishing large differences, the GC-MS method was adequate (3).

Blood pressure measurement

Home blood pressure

During each 1-wk intervention period, participants measured their blood pressure in the evening (2–3 h after dinner) by using an A&D Medical UA-767PC digital blood pressure monitor (A&D Instruments Ltd.). After a 5-min rest in a seated position, 5 blood pressure measurements were performed with a 1-min interval between measurements. The first reading was discarded, and an average of the remaining 4 readings was obtained.

Ambulatory blood pressure

On day 7 of each intervention period, a 24-h ambulatory blood pressure recording was performed. Ambulatory blood pressure was assessed by using a Spacelabs monitor (Spacelabs Medical Inc.) fitted by a trained researcher ~2.5 cm above the antecubital fossa on the nondominant arm. At 20-min intervals during the day and 30-min intervals during the night, blood pressure and heart rate were measured. Participants were instructed to continue their usual daily activities and to avoid vigorous physical activity. Excluded from the analysis were measurements that showed an error code or subjects with a pulse pressure <20 mm Hg. Blood pressure traces were considered complete if >80% of recordings were valid.

Urinary sodium, potassium, and creatinine analyses

Concentrations of urinary sodium, potassium, and creatinine were determined by using routine biochemical analyses performed by the Department of Clinical Biochemistry at Royal Perth Hospital. An ion-selective electrode with an automated analyzer (Roche Hitachi 917; Roche Diagnostics Australia Pty. Ltd.) was used for sodium and potassium analyses. Creatinine was measured by using a kinetic colorimetric test (Roche) with an automated analyzer (Roche Hitachi 917; Roche Diagnostics Australia Pty. Ltd.).

Other biochemical analyses

Routine biochemical analyses were performed at screening in the PathWest laboratory at Royal Perth Hospital, Western Australia. Serum total cholesterol, HDL cholesterol, and triglycerides were measured by using a routine enzymatic colorimetric test with a fully automated analyzer (Roche Hitachi 917; Roche Diagnostics Australia Pty. Ltd.). LDL-cholesterol concentrations were calculated by using Friedewald's formula (16). Serum glucose was measured by using an ultraviolet test with a fully automated analyzer (Roche Hitachi 917; Roche Diagnostics Australia Pty. Ltd.).

Statistics

The primary outcome for this study was blood pressure, which was measured as the mean 24-h day-time ambulatory blood pressure measured on day 7 of each intervention, and the mean home blood pressure, which was measured in the evening throughout each 7-d intervention. Our sample size was based on the primary outcome of the mean 24-h ambulatory blood pressure. At $\alpha = 0.05$, we estimated that 25 participants would provide >80% power to detect a 3-mm Hg difference in the mean 24-h ambulatory blood pressure. This sample size was also estimated to provide >80% power to detect a 3-mm Hg difference in mean home blood pressure. Statistical analyses were performed with SPSS 21.0 software (SPSS Inc.) and SAS 9.4 software (SAS Institute Inc.). Baseline participant characteristics are presented as means ± SDs and ranges. Categorical variables are summarized by the number in each category. Results in text and tables are presented as means ± SDs or means (95% CIs). Results in figures are presented as means ± SEs. Home and ambulatory blood pressures were analyzed by using mixed models in SAS 9.4 software by using the PROC MIXED command (SAS Institute Inc.). The subject was included as a random factor in each model. Fixed effects included the treatment (active or placebo), order, period, and hour (ambulatory blood pressure) or day (home blood pressure). Other outcome variables including plasma, salivary, and urinary nitrate and nitrite, and urinary sodium, potassium, and creatinine excretions were analyzed by using a paired samples *t* test with SPSS 21.0 software. A 2-sided type 1 error rate of $P < 0.05$ was the level of significance used for all hypothesis testing.

RESULTS

Baseline and descriptive data

The study was conducted from January 2013 to August 2013. Twenty-seven participants were randomly assigned (10 men and 17 women), and all participants completed the study (**Figure 1**). Data from all participants who completed the study were available for analysis. Participant characteristics are shown in **Table 1**. Participants took between 1 and 3 antihypertensive medications. Eight participants were taking one antihypertensive medication, 14 participants were taking 2 antihypertensive medications, and 5 participants were taking 3 antihypertensive medications. Ten participants were taking a calcium channel blocker, 6 participants were taking an angiotensin-converting enzyme inhibitor, 16 participants were taking an angiotensin II receptor antagonist,

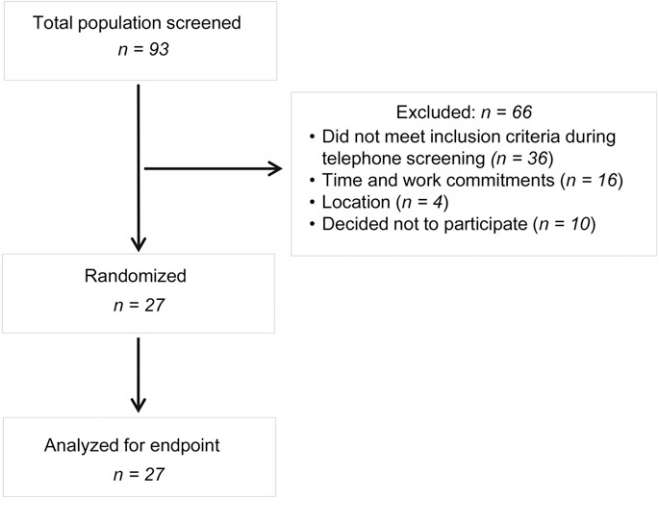

FIGURE 1 Participant flow from recruitment through screening and random assignment to trial completion.

TABLE 1
Baseline characteristics of study subjects (n = 27; men: n = 10; women: n = 17)

	Range (mean ± SD)
Age, y	53–70 (63.2 ± 4.4)
Height, cm	155–184 (170.4 ± 8.5)
Weight, kg	56.6–96.6 (78.1 ± 10.9)
BMI, kg/m^2	21.0–34.4 (26.9 ± 3.2)
Systolic blood pressure, mm Hg	120–160 (132.9 ± 11.8)
Diastolic blood pressure, mm Hg	48–94 (76.2 ± 10.4)
Heart rate, beats/min	48–96 (63.9 ± 9.5)
Total cholesterol, mmol/L	3.8–6.7 (5.2 ± 0.9)
HDL cholesterol, mmol/L	0.7–1.8 (1.3 ± 0.2)
Triglycerides, mmol/L	0.5–2.7 (1.1 ± 0.5)
Glucose, mmol/L	4.7–6.2 (5.5 ± 0.4)

6 participants were taking a β blocker, and 10 participants were taking a diuretic.

Dietary nitrate, plasma nitrite, and salivary and urinary nitrate and nitrite

The nitrate-rich beetroot juice (high nitrate) had 3.1 g nitrate/L (range: 2.3–3.9 g nitrate/L) and 0.1 g nitrite/L (range: 0.05–0.16 g nitrite/L). The nitrate-depleted beetroot juice (low nitrate) had 0.3 g nitrate/L (range: 0.04–0.5 g nitrate/L) and 0.006 g nitrite/L (range: 0.001–0.01 g nitrite/L). Therefore, during the high-nitrate intervention, participants increased their nitrate intake by 434 mg/d (range: 322–546 mg/d) and their nitrite intake by 14 mg/d (range: 7–22 mg/d). During the low-nitrate intervention, participants increased their nitrate and nitrite intakes by 42 mg/d (range: 5.6–70 mg/d) and 0.8 mg/d (range: 0.14–1.4 mg/d), respectively.

Plasma nitrite, salivary, and urinary nitrate and nitrite values post–low-nitrate and high-nitrate interventions are presented in **Table 2**. Plasma, salivary, and urinary nitrate and nitrite were significantly increased ($P < 0.001$) after the high-nitrate intervention than after the low-nitrate intervention. The high-nitrate intervention resulted in a 3-fold increase in both plasma nitrite and nitrate, a 7-fold increase in salivary nitrite, an 8-fold higher salivary nitrate, and a 4-fold increase in both urinary nitrite and nitrate than with the low-nitrate intervention.

Blood pressure

For 24-h ambulatory blood pressure, all blood pressure recordings for both interventions had >80% of the recordings valid, and all traces were considered complete. Blood pressure measurements during low- and high-nitrate interventions are presented in Table 2. Systolic blood pressure, diastolic blood pressure, pulse pressure, and heart rate measured by using home blood pressure (**Figure 2**) and ambulatory blood pressure (**Figure 3**) were not significantly different after the high-nitrate intervention than after the low-nitrate intervention.

Urinary sodium, potassium, and creatinine

Twenty-four-hour urinary sodium, potassium, creatinine, creatinine-corrected urinary sodium and potassium, and urinary sodium-to-potassium ratios at day 7 are provided in **Table 3**. There were no significant differences in sodium or potassium excretion between low- and high-nitrate intervention periods.

TABLE 2
Plasma, salivary, urinary nitrite and nitrate, SBP, DBP, PP, and HR for low- and high-nitrate interventions[1]

	Low-nitrate intervention	High-nitrate intervention	Difference	P
Nitrite[2]				
Plasma, μmol/L	2.0 ± 1.9	5.8 ± 2.9	−3.8 ± 2.6	<0.001
Salivary, μmol/L	112.3 ± 175.5	864.6 ± 439.0	−752.4 ± 295.3	<0.001
Urinary, μmol/L	98.3 ± 88.2	482.2 ± 190.0	−383.9 ± 155.6	<0.001
Nitrate[2]				
Plasma, μmol/L	36.2 ± 38.3	123.4 ± 53.3	−87.3 ± 48.8	<0.001
Salivary, μmol/L	257.1 ± 281.5	2241.1 ± 1153.8	−1984.0 ± 1023.8	<0.001
Urinary, μmol/L	350.2 ± 308.4	1430.0 ± 566.3	−1079.9 ± 451.4	<0.001
Home BP[3]				
SBP, mm Hg	127.5 (122.8, 132.2)	128.0 (123.3, 132.6)	−0.4 (−2.2, 1.3)	0.62
DBP, mm Hg	73.0 (69.8, 76.3)	72.7 (69.5, 75.9)	0.4 (−0.8, 1.5)	0.53
PP, mm Hg	54.5 (50.3, 58.7)	55.3 (51.1, 59.5)	−0.8 (−1.9, 0.3)	0.14
HR, beats/min	67.1 (64.7, 69.5)	67.7 (65.3, 70.1)	−0.7 (−1.6, 0.3)	0.17
Ambulatory BP[3]				
SBP, mm Hg	127.4 (123.0, 131.9)	128.4 (123.9, 132.8)	−0.9 (−2.1, 0.2)	0.08
DBP, mm Hg	73.8 (70.1, 77.6)	74.4 (70.6, 78.2)	−0.6 (−1.3, 0.2)	0.13
PP, mm Hg	53.6 (49.8, 57.4)	54.0 (50.1, 57.8)	−0.4 (−1.1, 0.3)	0.28
HR, beats/min	67.3 (64.6, 69.9)	68.2 (65.5, 70.9)	−0.9 (−1.7, −0.2)	0.01

[1] n = 27. BP, blood pressure; DBP, diastolic blood pressure; HR, heart rate; PP, pulse pressure; SBP, systolic blood pressure.

[2] All values are means ± SDs. Effects were analyzed by using paired samples tests with SPSS 21.0 software (SPSS Inc.).

[3] All values are means; 95% CIs in parentheses. Effects were analyzed by using mixed models with SAS 9.4 software (SAS Institute Inc.).

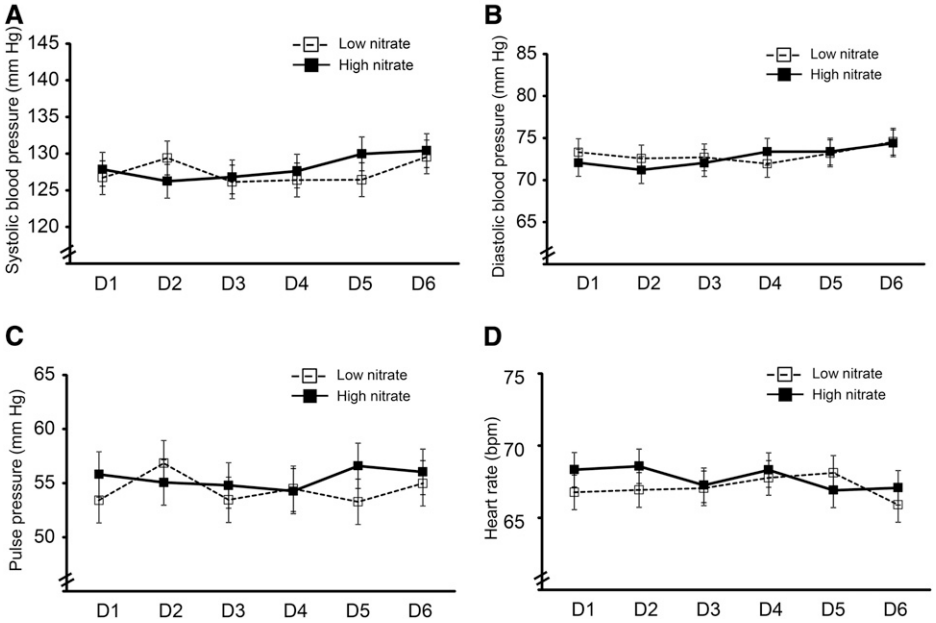

FIGURE 2 Mean ± SE effects of a 1-wk high-nitrate intervention compared with a 1-wk low-nitrate intervention in individuals with treated hypertension (n = 27) on home blood pressure–monitored systolic blood pressure (P = 0.62) (A), diastolic blood pressure (P = 0.53) (B), pulse pressure (P = 0.14) (C), and heart rate (P = 0.17) (D) from D1 to D6 of the intervention period. Effects were analyzed by using a mixed repeated-measures linear model in SAS 9.4 software (SAS Institute Inc.). bpm, beats per minute; D, day.

DISCUSSION

The effect of increased dietary nitrate intake from beetroot juice for 1 wk on blood pressure and nitrate metabolism in treated hypertensive men and women was assessed. Elevations in plasma, salivary, and urinary nitrate and nitrite indicated increased dietary nitrate intake and the potential for an increase in the physiologically relevant NO storage pool. However, this result did not translate into a reduction in blood pressure.

The randomized, controlled, crossover trial presented in this article has a number of key strengths. To our knowledge, only one previous study examined the effect of chronic dietary nitrate intake in treated hypertensives, which are a population at heightened risk of cardiovascular disease (5). The number of participants in the current study (n = 27) was high compared with other studies that investigated chronic nitrate intake on blood pressure; 9 of 13 studies conducted previously had participant

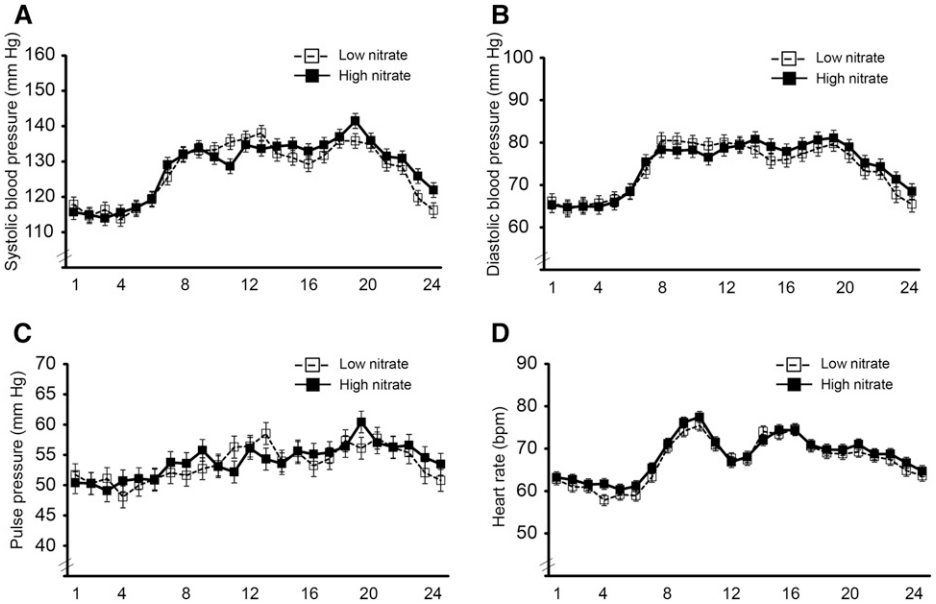

FIGURE 3 Mean ± SE effects of a 1-wk high-nitrate intervention compared with a 1-wk low-nitrate intervention in individuals with treated hypertension (n = 27) on 24-h ambulatory (A) mean hourly systolic blood pressure (P = 0.08) (A), mean hourly diastolic blood pressure (P = 0.13) (B), mean hourly pulse pressure (P = 0.28) (C), and mean hourly heart rate (P = 0.01) (D). Effects were analyzed by using a mixed repeated-measures linear model in SAS 9.4 software (SAS Institute Inc.). bpm, beats per minute.

TABLE 3
Twenty-four-hour urinary sodium, potassium, creatinine, and creatinine-corrected urinary sodium and potassium, and urinary sodium-to-potassium ratios at day 7 for low- and high-nitrate interventions[1]

	Low nitrate	High nitrate	*P*
Urinary sodium, mmol/d	130.3 ± 49.4	139.3 ± 48.9	0.41
Urinary potassium, mmol/d	97.3 ± 27.8	103.2 ± 31.6	0.24
Urinary creatinine, mmol/d	10.8 ± 3.0	11.6 ± 3.6	0.10
Urinary sodium, mmol/mmol creatinine	12.6 ± 5.7	12.5 ± 4.3	0.90
Urinary potassium, mmol/mmol creatinine	9.2 ± 2.0	9.1 ± 1.9	0.87
Urinary sodium-to-potassium ratio	1.4 ± 0.7	1.4 ± 0.4	0.73

[1]All values are means ± SDs. $n = 27$. Effects were analyzed by using paired samples tests with SPSS 21.0 software (SPSS Inc.).

numbers <12 (1, 5, 6). Blood pressure was rigorously assessed by using 2 clinically validated measurements that are independently predictive of risk of cardiovascular death (17). Ambulatory blood pressure was performed on day 7 of each intervention period. Home blood pressure monitoring was performed throughout the intervention periods. In addition, a placebo was used in the form of nitrate-depleted beetroot juice that was identical in taste and appearance to the nitrate-rich beetroot juice.

Participants increased their dietary nitrate intake from beetroot juice during the high-nitrate intervention by, on average, 434 mg/d (range: 322–546 mg/d). This is a dose with previously shown vascular benefits. Five of 6 previous chronic studies in healthy volunteers, with durations from 3 to 15 d, that investigated the effect of nitrate from beetroot juice observed a decrease in blood pressure with doses that ranged from 316 to 595 mg nitrate (3). Although the majority of studies used beetroot juice as a nitrate source, other chronic studies used green leafy vegetables and sodium nitrate. Sobko et al. (18) observed a significant decrease in diastolic blood pressure with 10 d intake of high nitrate vegetables present in a traditional Japanese diet (±1200 mg/d). In 2 separate studies by Larsen et al. (19, 20), a significant decrease in blood pressure was observed with 3 d intake of ±400 mg $NaNO_3$. Therefore, intake of ∼400 mg nitrate/d in the current study was within the range previously shown to have chronic effects in healthy men and women.

Increases in plasma, salivary, and urinary nitrate and nitrite after 1 wk beetroot-juice intake were indicative of increased nitrate metabolism through the enterosalivary nitrate-nitrite-NO pathway and the elevation of molecules with the potential to be converted to NO (19, 21). Although values in the current study fell within the range of those observed in other studies, because of different analytic approaches, it was impossible to make a direct comparison. The high concentration of plasma nitrite after the low-nitrate intervention was most likely an artifact of the GC-MS method. Because the nitrate intervention increased plasma nitrate and nitrite 3-fold, this result suggested that participants consumed a low-nitrate diet, and the results were not due to a high–background nitrate diet. Because of differing analytic approaches, an incomplete understanding of the biology of nitrate, nitrite, and NO as well as probable laboratory nitrite contamination, the observed increases in circulating nitrate and nitrite were not a direct measurement of NO bioavailability. However, plasma nitrite is associated with measures of vascular function and can be readily reduced to NO. How this reduction occurs is unclear but can potentially occur via a number of mechanisms including reactions with hemoglobin, myoglobin, and xanthine oxidoreductase as well as acidic reduction (22). The reduction of nitrite to NO is now recognized to be an important pathway that regulates basal vascular tone, arterial stiffness and, therefore, blood pressure. Indeed, improvements in measures of vascular function such as arterial stiffness and blood pressure were observed with parallel increases in plasma nitrite after dietary nitrate consumption (15, 23).

In this study of treated hypertensive participants, home-monitored blood pressure and ambulatory blood pressure were unchanged after 7-d intake of nitrate-rich beetroot juice despite parallel increased nitrate metabolism. In addition, the timing of the home blood pressure measurements (∼2 h after the evening nitrate dose) indicated a lack of any repeated acute blood pressure reduction. These results contrast with previous reports in the literature, in particular with results shown in healthy volunteers. However, in another cohort at high risk of cardiovascular disease, individuals with type 2 diabetes, Gilchrist et al. (7) observed no effect on blood pressure or endothelial function with 14 d supplementation of 465 mg nitrate from beetroot juice. In addition, we recently showed that a short-term increase in nitrate derived from green leafy vegetables of ∼400 mg/d did not result in lower blood pressure in a group with blood pressure in the high-normal range (3). In contrast, Rammos et al. (8) showed a reduction in systolic blood pressure and an improvement in endothelial function with 900 mg $NaNO_3$ supplementation for 28 d in 11 elderly volunteers with a moderate risk of cardiovascular disease. Jajja et al. (6) observed a significant reduction in systolic blood pressure measured by using home blood pressure monitoring in the third week of dietary nitrate (165 mg from beetroot juice) in 10 older, overweight subjects with high-normal blood pressure. No effect was observed in blood pressure measured by using clinic or ambulatory blood pressure. In a recent study by Kapil et al. (5), daily intake of dietary nitrate (±400 mg from beetroot juice for 4 wk) by drug-naive and treated hypertensives resulted in a significant reduction in systolic blood pressure as measured by clinic, home, and ambulatory blood pressure monitorings.

Hypertension is a multifactorial disease and results from genetic, physiologic, and environmental interactions. Although numerous factors have been implicated in its pathophysiology, a common pathway is thought to be a loss of normal endothelial function (24). A normal endothelium vasodilates in response to the secretion of molecules such as NO, prostacyclin, and endothelial-derived hyperpolarizing factors; however, the principal mediator is NO (24). An increase in the circulating NO pool through the enterosalivary nitrate-nitrite-NO pathway improves endothelial function and decreases blood pressure in healthy individuals (1). A similar increase in the circulating NO pool with dietary nitrate intake in treated hypertensives in this study did not have the same effect on blood pressure.

There are a number of possible explanations for the lack of effect on blood pressure of increased nitrate intake in the treated hypertensive individuals in this study. Many of the pharmacologic agents currently used for the treatment of hypertension influence NO production (25). Because all our volunteers were taking between 1 and 3 medications for hypertension, this intake may have influenced the response to dietary nitrate. It could also be argued that no additional substantial fall in blood pressure

might be anticipated in a group of subjects whose hypertension was already relatively well controlled on medication. The baseline blood pressure of volunteers in this study was lower than in those in the study by Kapil et al. (5), which indicated that their blood pressure was already well controlled. However, in several previous trials in similarly treated hypertensives, we showed clinically significant additional falls in blood pressure with diet or lifestyle interventions (26–28). Another possible explanation was the length of the dietary nitrate intervention. Jajja et al. (6) observed a significant reduction in blood pressure in week 3 of dietary nitrate intake, but not weeks 1 and 2, in individuals with high-normal blood pressure. Kapil et al. (5) showed a significant reduction in systolic blood pressure that persisted throughout the entire 4 wk of dietary nitrate supplementation in treated and drug-naive hypertensive patients. Rammos et al. (8) observed significant reductions in systolic blood pressure in individuals with mild hypertension after 4 wk of nitrate intake. We observed no effect on blood pressure after 1 wk of dietary nitrate intake in individuals with high-normal blood pressure and treated hypertensives in this study. Gilchrist et al. (7) observed no effect on blood pressure after 2 wk of dietary nitrate intake in individuals with type 2 diabetes and similar blood pressures. However, studies in healthy individuals observed significant reductions with 6–15 d of dietary nitrate intake (1). Finally, there was also a possible cross-talk between the enterosalivary nitrate-nitrite-NO pathway and the L-arginine-nitric oxide synthase pathway. Although it has yet to be confirmed in human studies, evidence suggested that increasing NO through one pathway could downregulate NO production through the other pathway with a net zero effect (29).

In conclusion, we observed that regular intake of dietary nitrate from beetroot juice for 1 wk had no effect on blood pressure in treated hypertensive men and women. Contrary to evidence from clinical studies that investigated regular intake of dietary nitrate in healthy individuals, these results suggest that increasing dietary nitrate may not be an effective strategy to reduce blood pressure in drug-treated hypertensive men and women. Future studies are required to confirm this finding and in other populations at heightened risk of cardiovascular disease.

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