Dietary Nitrate Supplementation Attenuates Blood Pressure in Young Prehypertensive Men during Exercise

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Abstract

Background: Acute dietary nitrate (NO_3-) supplementation with beetroot juice (BRJ) can lower blood pressure (BP) at rest and during exercise in healthy individuals; however, the effects on endothelial function and BP response to dynamic exercise are not known in prehypertensive individuals. We compared the effects of 15 days BRJ supplementation on hemodynamic responses during progressive dynamic exercise.

Methods: In a double-blind, randomized, crossover design, 11 healthy, prehypertensive men were supplemented with either BRJ (5.6 mmol, 70 ml BRJ) or a placebo (PL)(70 ml control drink) every day for 15 days. Participants completed two bouts of cycling exercise at each of the two workout intensities, corresponding to 30% and 60% of their predetermined VO_{2peak} values. Flow-mediated dilation (FMD) of the brachial artery and plasma concentration of NOx (NO_3- and NO_2-) were measured, and the mean arterial pressure (MAP), cardiac output (CO), and total vascular conductance (TVC) were assessed at rest and during exercise before and after each treatment.

Results: BRJ supplementation significantly increased resting plasma NOx concentrations (123.0±11.3 vs. 181.9±19.5 μM) and the brachial artery FMD (9.8±1.0 vs. 13.5±1.4%) compared to no change after ingestion of the PL. Compared with the PL, BRJ supplementation reduced the MAP (101±1 vs. 99±1 mmHg) at rest and this reduction occurred across workloads, while the TVC was increased only during exercise ($p<0.05$). There was no difference in CO.

Conclusions: 15 days of dietary nitrate supplementation could improve endothelial function and contribute to attenuation of an exaggerated exercise BP resulting mainly from a failure to reduce peripheral resistance during exercise.

Keywords: Beetroot juice; Dynamic exercise; Cardiac output; Endothelial function

Introduction

Prehypertension is defined as a resting systolic blood pressure (SBP) between 120 and 139 mmHg and/or a diastolic blood pressure (DBP) between 80 and 89 mmHg. This definition was introduced in the Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7).[1] This new category was created to emphasize the potential risk of blood pressure (BP) in this range for future development of clinical hypertension. Long-term exercise training might provide protective effects by lowering BP. Accordingly, the JNC 7 recommends that individuals with prehypertension participate in regular physical activity, as an alternative to drug therapy, in order to prevent the progressive increase in BP.[1] However, cardiac events associated with exercise, such as stroke and acute myocardial infarction, have been reported in individuals with prehypertension.[2] These events might be caused by excessive increases in both BP and heart rate (HR) during exercise.[3,4]

A recent investigation reported a BP response to exercise in participants with prehypertension and found that increases in BP evoked by forearm static contraction were augmented in those with prehypertension compared with those with a normal BP.[5] This phenomenon was associated with elevated vasoconstriction (i.e., increased peripheral resistance). Previous studies have provided evidence that impaired endothelium-dependent vasodilation is associated with prehypertension[6] and evokes an exaggerated BP response during exercise in individuals with high-normal BP or mild hypertension.[7] Consequently, the endothelium may be a possible target for the preventive intervention of prehypertension.

It is well-known that nitric oxide (NO), which is produced endogenously by the reduction of L-arginine to L-citrulline via NO synthase, plays an important role in the regulation of skeletal muscle vascular control.[8,9] Dietary nitrate supplementation is also regarded as an alternative source of NO. Nitrate (NO_3-) is abundant in green leafy vegetables and beetroot (BR)[10] and is converted to a nitrite anion (NO_2-) in the mouth via facultative anaerobic bacteria on the surface of the tongue and, subsequently, to NO and other reactive nitrogen intermediates.[11] The reduction of NO_2- to NO is facilitated by hypoxic conditions,[12,13] which can occur during exercise.

Previous studies have indicated that acute and chronic dietary nitrate supplementation with BR juice substantially elevated plasma NO_3-/NO_2- (NOx) and reduced BP in healthy individuals at rest and during exercise. It has been suggested that this effect is likely due to a decrease in systemic vascular resistance rather than in cardiac output (CO).[14,15] It is possible that increased plasma NO_2- increases NO bioavailability, resulting in enhanced smooth muscle relaxation via the synthesis of cyclic guanosine monophosphate from guanosine triphosphate, therefore being partially responsible for vasodilation and a reduction in systemic vascular resistance during exercise.[16,17] Thus, due to the fact that impaired endothelial function and exercise hyperemia contribute to an exaggerated blood pressure response,[7] improvement in vascular endothelium function via dietary nitrate supplementation might attenuate the BP response to exercise in individuals with prehypertension. We hypothesized that nitrate-containing BR juice would improve brachial artery flow mediated dilation (FMD) and exercise-induced vasodilation, as demonstrated by a decrease in peripheral vasoconstriction during dynamic exercise, and thus reduce the exercise-induced increase in BP.

Methods

For this study, 11 healthy voluntary men, aged 20 to 24 years, were recruited from students on a university campus and the surrounding community using the advertisement flyers. Prior to testing, informed consent was obtained from each participant. All procedures were reviewed and approved by the KyungHee University Institutional Review Board (KHU 2012-03). The inclusion criteria for prehypertensive individuals were SBP of 120 to 139 mmHg and/or DBP of 80 to 89 mmHg.[1] Individuals using antihypertensive medication were excluded. All participants were sedentary and free from any signs or symptoms of overt coronary heart disease (based on health history questionnaires and resting electrocardiograms) and were considered to be in good health. Participants were instructed to adhere to their normal living routine and diet throughout the experiment. Participants completed one maximum graded exercise test and two submaximal tests and were instructed to avoid intense physical activity for 48 hours prior to each testing session. Each exercise test was performed at the same time of day for each participant. Participants were asked to refrain from consuming alcohol or caffeine-containing beverages for at least 48 hours prior to each test.

Exercise Protocols

Resting BP was measured in the brachial artery in a seated position. After 5 minutes of sitting rest, 3 measurements were obtained 5 minutes apart, using a sphygmomanometer. Subjects who met the criteria for three measurements qualified for this study. BP was expressed as the average of the 3 measurements. The resting and exercising BP were measured in the left brachial arterial by a well-trained investigator. To determine the relative exercise intensities of the two workloads used in the present study (30% and 60% VO_{2peak}), a maximal exercise test was performed on a cycle ergometer (Monark 828, Sweden). The protocol began with 2 minutes of unloaded baseline cycling followed by increases of 30 watts every minute until participants could no longer maintain a pedal cadence of 60 rpm. The breath-by-breath pulmonary gas exchange data were continuously measured with an Ultima CPX Metabolic Measurement Cart (Medgraphic, St. Paul, Minnesota, USA). The VO_{2peak} obtained from this test was used as an index of functional capacity. The participants then completed 2 bouts of exercise at the constant submaximal workloads corresponding to 30% and 60% of their predetermined VO_{2peak} values, with every workload lasting 5 minutes. Participants completed a progressive exercise test (30% and 60% VO_{2peak}) on the same day.

Supplementation

Following completion of the submaximal tests, a double-blind, randomized, cross-over study was performed in order to test the effects of dietary supplementation containing beetroot juice (BRJ) (5.6 mmol administrated as 70 ml BR juice/day) (Saengdr Agriculture CO, Jeju island, Korea) compared with the placebo (PL) (70 ml water/day). The PL water was colored to look identical to the BRJ in a double-blind experimental design. Eleven participants received 15 days of dietary supplementation with either BRJ or PL. An at least 2-week washout period separated each supplementation period. Participants were asked to maintain their normal daily activities and food intake during the entire study period. The investigator administering the exercise tests was not aware of the type of beverage being consumed by the participants.

Flow-Mediated Dilation (FMD) Study

Vascular measurement was performed in a quiet room at approximately 22°C. The effect of BRJ on endothelial function in response to reactive hyperemia was assessed in participants by measuring the brachial artery diameter in the right arm using a high-resolution ultrasound machine (ClearVue 550, USA) equipped with a 12 MHz transducer. The images were obtained and analyzed by the same examiner in a blinded manner. The baseline end diastolic brachial diameters and blood velocity were measured with the transducer placed 3 to 5 cm in the antecubital fossa above its bifurcation. To produce reactive hyperemia, the pressure cuff placed on the upper arm was inflated by 200 mmHg for 5 minutes, followed by a rapid deflation, and the brachial artery was imaged and recorded for 2 minutes. The absolute change in diameter was determined, and the FMD was expressed as the highest percentage change (%FMD) in brachial diameter from baseline.

Measurement of Hemodynamic Variables

Impedance cardiography (Physio Flow, Manatec Biomedical, France) was used in the present study to continuously measure stroke volume (SV) and HR. The device provides real-time CO data and measures cardiac parameters in healthy participants.[18,19] The bioimpedance device consists of 2 impedance cardiography electrodes placed above the supraclavicular fossa at the base of the left side of the neck, 2 electrocardiography electrodes used for recording the ECG, and 2 electrodes placed at the xiphoid process. The Physioflow measures the change in transthoracic impedance during the cardiac cycle. CO was calculated according to the following formula: CO = HR x SVi x BSA, where HR was measured from the R-R interval determined from the first derivative of the ECG, SVi is the SV index (i.e., SV/BSA), and BSA (body surface area) (m^2) was determined according to the Haycock formula: $BSA = 0.024265 \times BM^{0.5378} \times H^{0.3964}$, where BM is the body mass in kilograms, and H is the height in centimeters. The Physioflow has previously been validated and was found to be highly correlated with the direct Fick method at rest and during submaximal and maximal exercise.[19,20]

The BP during exercise was measured in the left brachial artery of using a sphygmomanometer under steady-state conditions, by the same investigator in each participant throughout the experiment. The MAP was calculated using the formula: MAP = [(SBP-DBP) x 1/3] + DBP. Total vascular conductance (TVC) was calculated as CO/MAP. The rate-pressure product (RPP) was calculated as HR x SBP.

Measurement of Plasma NOx

To collect blood samples from the placebo- and beetroot-

supplemented groups for the measurement of plasma [NO_3-] and [NO_2-], a catheter with a 2-gauge needle was inserted into the brachial vein. Then, 5 ml venous blood was drawn at rest before and after dietary PL and BRJ supplementation. Samples were centrifuged at 3,000 rpm for 10 minutes, and the plasma fractions were subsequently isolated and immediately frozen at -80°C until the analysis of [NO_3-] and [NO_2-].

NO in the plasma was assessed by measuring the levels of nitrate plus nitrite (NOx), an oxidative metabolite of NO, using a colorimetric assay with Griess Reagent in a microtiter format (Cayman, Ann Arbor, Michigan, USA). Spectrophotometric quantitation of nitrite based on the Griess Reagent is straightforward, and the NADH-dependent enzyme nitrate reductase was used to convert nitrate to nitrite prior to quantitation using Griess Reagent. The intraassay and interassay coefficients of variation for the NOx measurement with this method were 5.2% and 6.6%, respectively.

Table 1. Physical characteristics of subjects.

Variables	Participants (*n*=11)
Age (yrs)	23±1
Height (cm)	178.7±1.2
Body weight (kg)	81.1±3.3
BMI (kg/m^2)	25.4±0.9
SBP (mmHg)	132±1
DBP (mmHg)	86±1
MAP (mmHg)	101±0
Resting HR (beats/min)	70±3
VO_{2peak} (ml/kg/min)	40.0±1.4

Values are expressed as the mean ± standard error; BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, HR: heart rate, VO_{2peak}: peak oxygen uptake.

Table 2. Effects of dietary nitrates on plasma NOx, baseline diameter, maximal diameter, and (%) FMD at rest.

	PL		BRJ	
	Pre	Post	Pre	Post
NOx (μM)	120.0±9.1	130.2±13.7	125.2±9.6	182.2±17.9*
Baseline diameter (mm)	4.66±0.22	4.71±0.15	4.51±0.16	4.58±0.17
Maximal diameter (mm)	5.11±0.24	5.13±0.18	4.96±0.19	5.19±0.18*
(%) FMD	9.69±1.48	8.93±1.62	9.82±0.99	13.48±1.35*

Values are expressed as the mean ± standard error; BRJ: beetroot juice, PL: placebo water; NOx: nitrate + nitrite, FMD: flow mediated dilation.
*$p < 0.05$, vs. pre.

Data Analysis

It has been reported that the SV measurements using impedance cardiography under steady-state conditions are reliable and valid in healthy participants.[18,19] Therefore, this device accurately measures the absolute values of SV at rest and during submaximal exercise. To determine the effects of dietary nitrate on cardiovascular responses during exercise, the values were expressed as absolute values. The mean values of HR, SV, and CO at each 30-second interval at rest and during exercise were used for comparative purposes. BP was measured between the fourth and fifth minutes of each workload, immediately prior to blood collection. To compare the effects of BRJ over workloads and between groups, two-way repeated-measures ANOVA and Tukey's post hoc test were used. Mean values of all variables between groups were compared using an independent Student's *t*-test, and a Student's paired *t*-test was used to compare the mean differences between placebo and beetroot supplementation. The significance level was set at $P < 0.05$.

Results

Physical characteristics of the subjects are presented in Table 1. The subjects that participated in this study were considered as "prehypertension" according to the norms set forth in the Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7).[1] VO_{2peak} values indicated that the cardiorespiratory fitness were within the normal range for this group.

Table 2 shows the effects of BRJ supplementation on plasma NOx (NO_3-/NO_2-) concentration and brachial artery diameter. BRJ supplementation significantly increased the plasma concentration of NOx at rest ($P < 0.05$). There were no significant differences in baseline diameter between the two conditions, while BRJ significantly increased maximal diameter ($P < 0.05$). Maximal FMD as a percentage change in artery diameter significantly increased after BRJ supplementation ($P < 0.05$). PL supplementation had no effect on either the NOx concentrations or the baseline/maximal diameters.

Figure 1 shows the absolute values of the hemodynamic variables at rest and across workloads between pre-PL

Figure 1. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR), rate pressure product (RPP), stroke volume (SV), cardiac output (CO), and total vascular conductance (TVC) at rest and across workloads between pre-PL and pre-BRJ supplementation conditions.

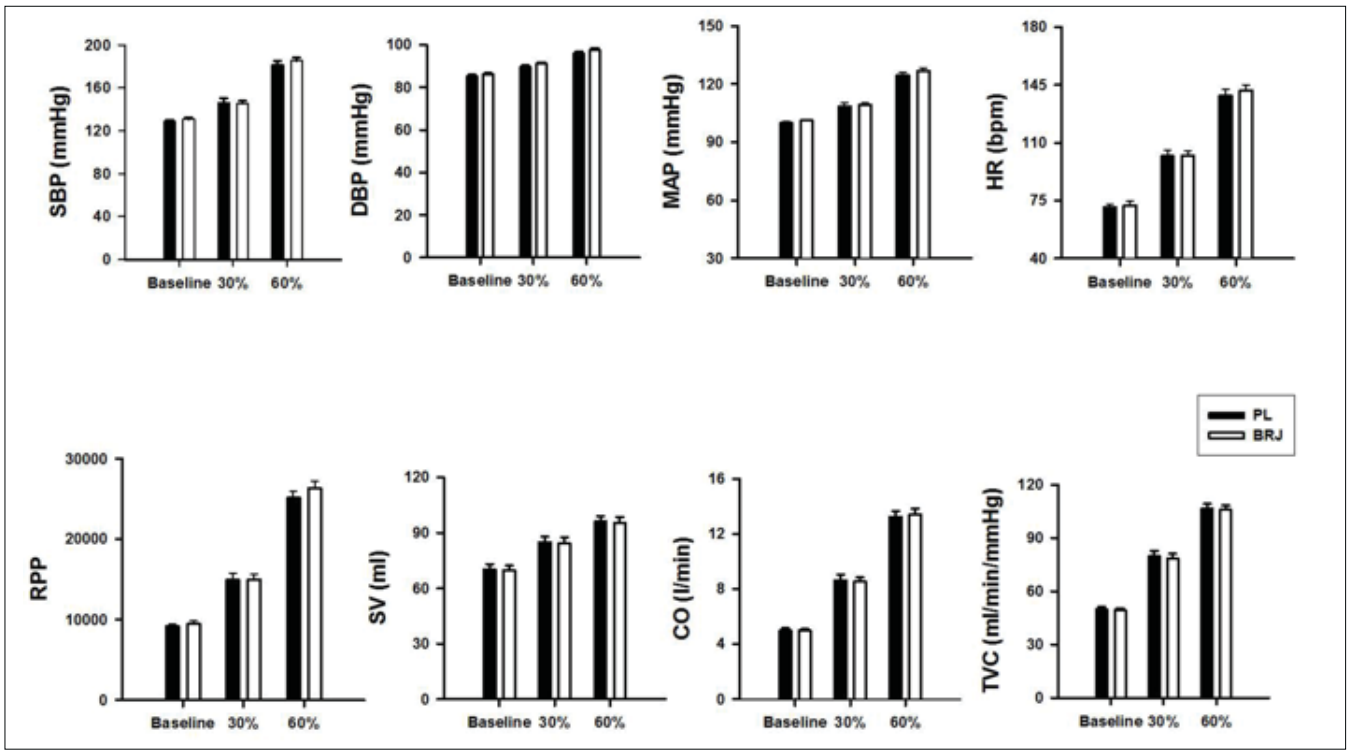


Figure 2. SBP, DBP, MAP, HR, RPP, SV, CO, and TVC at rest and across workloads following supplementation with PL.

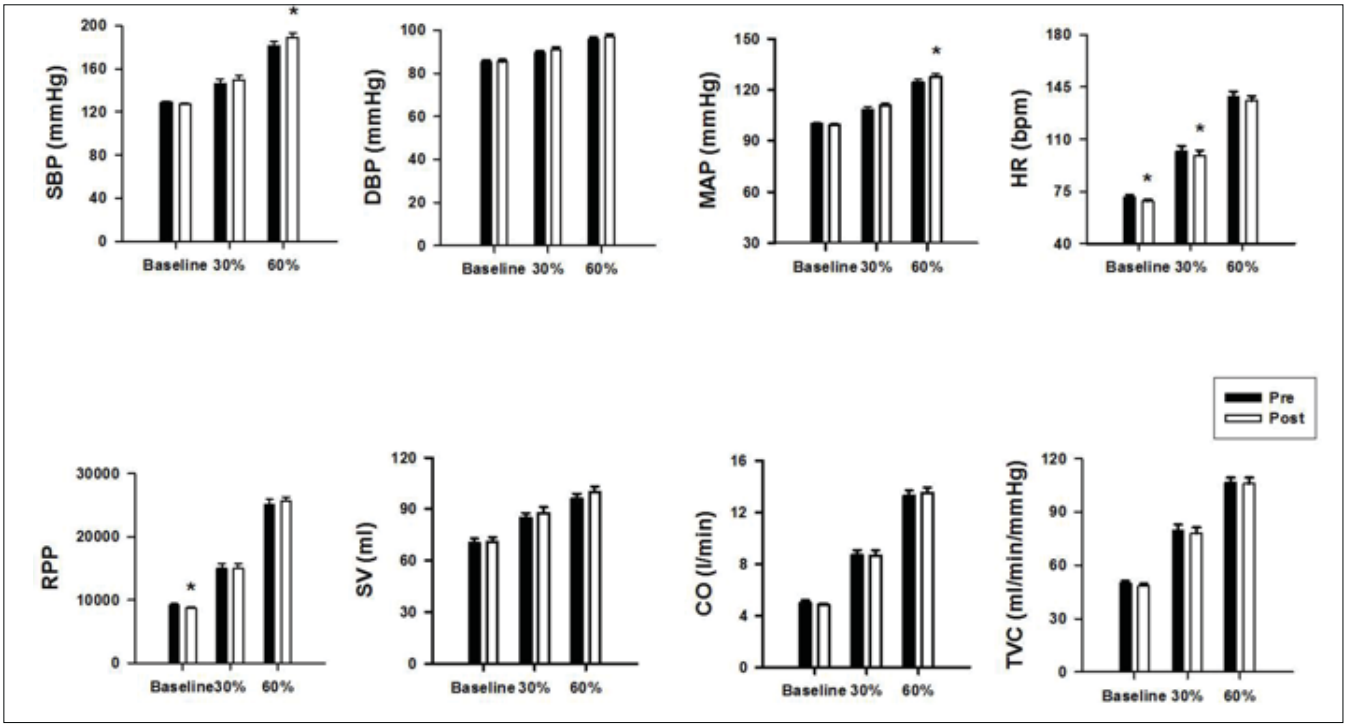
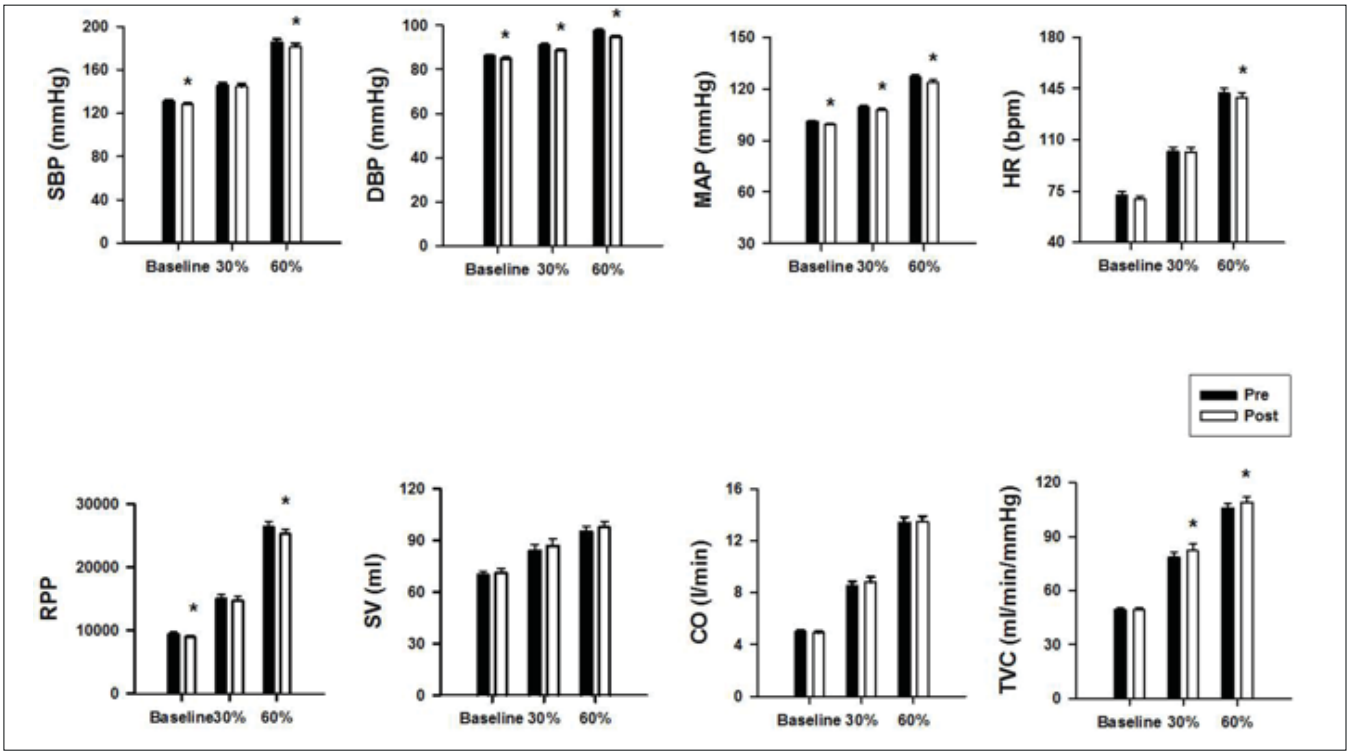


Figure 3. SBP, DBP, MAP, HR, RPP, SV, CO, and TVC at rest and across workloads following supplementation with BRJ.



and pre-BRJ supplementation conditions. The values of all hemodynamic variables were similar at rest and during exercise when compared for the two conditions.

Figure 2 shows the absolute values of the hemodynamic variables at rest and at the constant workloads corresponding to 30% and 60% of the predetermined VO_{2peak} before and after PL supplementation. HR was significantly lowered at rest and at 30% workload ($P < 0.05$), and RPP was significantly lowered at rest ($P < 0.05$). No statistically-significant differences in SBP, DBP, MAP, SV, CO, or TVC were altered by PL supplementation.

Figure 3 shows the absolute values of the hemodynamic variables at rest and at the constant workloads corresponding to 30% and 60% of the predetermined VO_{2peak} before and after BRJ supplementation. BRJ significantly reduced SBP, DBP, and MAP at rest and during all workloads, while TVC was increased at every workload ($P < 0.05$). There were no significant differences in either SV or CO at rest or at either workload after BRJ supplementation. RPP was significantly lowered at rest and at 60% workload, although there was a tendency of RPP to decrease at 30% workload (P=0.09). No effects of BRJ on HR were observed. There were significantly positive correlations between NOx and FMD before and after BRJ supplementation (r = 0.46, $P<0.05$).

Discussion

This is the first study to evaluate the effects of chronic dietary nitrate supplementation on MAP, CO, and TVC in response to dynamic exercise and on brachial artery FMD in young individuals with prehypertension. The new findings of this investigation show that 15 days of dietary BR supplementation in individuals with prehypertension substantially increased plasma NOx concentration, leading to a significant reduction in MAP and an increase in TVC at rest and across workloads. There were no significant differences in CO between the two conditions. Endothelial function was significantly improved after dietary nitrate consumption. Thus, elevated plasma NOx resulting from BRJ intake led to a significant reduction in MAP via an increase in peripheral vascular conductance during dynamic exercise.

Effect of BRJ Supplementation on Brachial Artery Endothelial Function

In the present study, we found that dietary nitrate supplementation improved endothelial function by approximately 38%, with no changes seen after PL supplementation. Beck et al.[23] recently evaluated endothelial function in individuals with prehypertension and reported that plasma concentration of NOx was significantly lower than that of normotensive individuals, and that impaired endothelial function was correlated with reduction in the plasma level of NOx. Accordingly, it is likely that the increased plasma NOx bioavailability via BRJ supplementation observed in our study improved the dysfunction of the vascular endothelium in the individuals with prehypertension. Taken together, these findings suggest that dietary nitrate supplementation has beneficial effects on endothelial function in young individuals with prehypertension.

Effect of BRJ Supplementation on Resting and Exercising BP

The current study demonstrates that chronic dietary nitrate intake leads to a significant reduction in SBP, DBP, and MAP at rest and during exercise in sedentary individuals with prehypertension compared with the PL control group. There is evidence that both acute and chronic dietary nitrate intake via BRJ increased plasma nitrite concentration, reducing the resting BP in healthy individuals.[21,22] A recent study examined the extent to which acute BP supplementation attenuated the increase in BP response at 40%, 60%, and 80% VO_{2peak} workloads in healthy female participants and reported that the SBP was lowered across workloads.[14] An animal study also showed that five days of BR supplementation in healthy rats lowered exercising MAP.[15] However, these previous studies only measured the changes in BP in healthy subjects. We extend these previous studies by demonstrating that dietary nitrate supplementation reduces the BP response in pre-hypertensive individuals during exercise.

Effect of BRJ Supplementation on CO and Peripheral Vasodilation

Dysfunction of endothelium-dependent vasodilation is mainly induced by a reduction in the bioavailability of endothelium-derived NO_2[4] and is associated with prehypertension and hypertension.[6,25] Impaired endothelial vasodilation in the smaller resistance vessels might contribute to an exaggerated BP response to exercise.[26] In a previous work, the reflex pressor response to exercise has been shown to be augmented in prehypertension, and this phenomenon results primarily from a failure to reduce total peripheral resistance during exercise. Conversely, other studies have demonstrated that dietary nitrate supplementation lowered blood pressure and improved endothelial function in healthy individuals.[27,28] The present study demonstrated significant systemic vasodilation during exercise, likely attributed to an improvement in endothelial function via BRJ supplementation.

It appears that nitrate supplementation-induced attenuations in arterial BP were associated with concomitant attenuations of exercise vasodilation, suggesting that the afterload on the heart was reduced and this contention is supported by the fact that RPP, an indicator of myocardial oxygen demand, was also attenuated. A decrease in two variables would be expected to increase in CO. However, the corresponding reduction of afterload induced by an increase in NO bioavailability at each workload may not have been sufficient to allow SV to increase. Taken together, these findings suggest that in pre-hypertensive men, despite the inability to increase CO, the dietary nitrate supplementation produced a decrease in BP via peripheral vasodilation at rest and during exercise.

Potential Limitation

Increases in BP in response to exercise are mediated by increases in sympathetic nerve activity to the heart and peripheral vasculature,[29] and studies have provided evidence that the sympathetic outflow is augmented in individuals with prehypertension.[30,31] This effect might result from increases in afterload via enhanced vasoconstriction. One potential mechanism of the BRJ-mediated reduction in MAP is inhibition of central sympathetic outflow from the brainstem by NO in both humans and animals. Previous studies have demonstrated that systemic administration of an NOS inhibitor in healthy individuals resulted in sympathetic activation and a substantial increase in blood pressure.[32,33] These findings imply that reduced central NO bioavailability increases sympathetic nerve activity (SNA). However, although NOx concentration was substantially increased at rest after BRJ supplementation, SNA was not measured in the present study, which is a limitation. In contrast, another study showed that the NO synthase inhibitor L-NAME enhanced the vasoconstrictor response to the same level of sympathetic

activation.[34] Accordingly, we may speculate that increased NO bioavailability via dietary nitrate supplementation decreases the vasoconstrictor response without altering the level of SNA, resulting in an attenuated BP response.

Perspectives and Significance

Our results have clinical implications for those who need to lower their BP because dietary nitrate supplementation can reduce both resting and exercising BP. Cardiac events associated with exercise, such as stroke and acute myocardial infarction, have been reported in both prehypertensive and hypertensive individuals, and these events are evoked by excessive increases in both BP and HR.[2] Since the enhanced BP response to exercise is related to endothelial dysfunction and increases in peripheral vasoconstriction, the addition of nitrate-rich vegetables to the normal diet may offer a beneficial effect on vascular tone and improve systemic hemodynamics during exercise.

Conclusion

We demonstrated that chronic dietary nitrate supplementation increases NO bioavailability, improves brachial endothelial function, and reduces resting and exercising BP in individuals with prehypertension who are at risk for development of hypertension and exercise hypertension. These observations suggest that the attenuated BP response to exercise is induced by a reduction in peripheral vasoconstriction associated with improvements in endothelial function.

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Conflict of Interest

The authors declare that they have no conflict of interest. This manuscript represents original research that has not been for publication elsewhere.

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