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Sodium Nitrate Supplementation Improves Blood Pressure Reactivity in Patients with Peripheral Artery Disease

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Abstract

Background & Aims: Peripheral artery disease (PAD) is characterized by elevated blood pressure (BP), low nitric oxide availability (NO), and exaggerated pressor responses to sympatho-excitatory stressors. Inorganic nitrate reduces peripheral BP in healthy and chronically diseased populations. The objective of this study was to investigate the effects of eight-weeks of sodium nitrate ($NaNO_3$) supplementation on indices of BP in PAD patients.

Methods & Results: 21 patients with PAD were recruited to participate in this study, undergoing 8-weeks of $NaNO_3$ (n=13; 73±9 years) or placebo (n=8; 69±10 years) supplementation. BP responsiveness to a cold pressor test (CPT) were examined prior to and following the supplementation period. The systolic BP response (change from rest) during the first (26±10 vs. 19±11mmHg) and second minutes (32±10 vs. 26±12mmHg) of CPT were reduced following $NaNO_3$ ($P<0.05$ for both) but not after placebo (first minute: 22±10 vs. 24±10mmHg, P=0.30; second minute 26±10 vs 27±10mmHg, P=0.72) supplementation.

Conclusion: Our data suggest that eight-weeks of $NaNO_3$ supplementation reduces BP responsiveness to sympatho-excitatory stimuli.

Clinical Trials Registration Number: NCT01983826

Keywords

Peripheral Arterial Disease; Inorganic Nitrate; Blood Pressure; Nitric Oxide

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Competing Interests

The authors have no competing interests or conflicts to report.

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Introduction

Peripheral arterial disease (PAD), characterized by systemic atherosclerosis, is associated with elevated blood pressure (BP), and low nitric oxide (NO) bioavailability.[1–3] Adequate BP management is crucial in these patients to reduce the risk of major adverse cardiovascular events (MACE).[4, 5] While static (in-office/clinic) BP is used to diagnose hypertension, BP responsiveness to physiological or psychological stressors are stronger predictors of future hypertension [6–10]

Supplementation with inorganic nitrate NO_3^- effectively reduces clinic/office BP.[11, 12] BP reduction via inorganic NO_3^- presents a new avenue in the management of BP in subjects with PAD. It is unclear whether inorganic NO_3^- supplementation improves BP responsiveness in patients with PAD. We hypothesized that $NaNO_3$ supplementation would minimize the exaggerated BP response to sympatho-excitatory stimuli.

Methods

Study Population

Twenty-one patients with PAD participated in the study. Inclusion criteria were clinically diagnosed PAD and the ability to walk without severe leg symptoms. Exclusion criteria included non-atherosclerotic vascular disease, critical limb ischemia, active ischemic ulceration, recent (within six months) revascularization, symptomatic coronary artery disease, heart failure, renal disease, hypotension (resting systolic BP <90mmHg), smoking or history of smoking within one year, and the use of phosphodiesterase V inhibitor drugs. All women enrolled in the study were postmenopausal and not receiving hormone therapy. All subjects completed written, informed consent to participate in study protocols approved by the Institutional Review Board at the University of Iowa. This study was a Registered Clinical Trial (reference identification NCT01983826) with some of the data (e.g., demographics, resting BP, plasma [NO_3^-] and [NO_2^-]) reported previously.[13, 14]

Subjects were randomized to either 1g per day of sodium nitrate ($NaNO_3$) or placebo (microcrystalline cellulose) supplementation for eight weeks using a 4:2 block design favoring $NaNO_3$. All investigators were blinded to group randomization. Subjects were requested to follow a low-nitrate diet for the three days preceding all visits, refraining from exercise, alcohol, and caffeine for 24-hours before reporting to the laboratory. Subjects visited the clinical research unit at the University of Iowa Hospital and Clinics for a venous blood draw and then the laboratory for assessment of BP responsiveness to a cold pressor test (CPT).

Blood Pressure Responsiveness

After 10 minutes of seated quiet rest, the left hand of the subject was immersed in ice water (0–4°C) for two minutes. After two minutes of immersion, the hand was placed on a towel for a recovery period of three minutes. Systemic BP was assessed (beat-to-beat) via finger plethysmography (Nexfin, Edwards Lifesciences, Irvine, CA) over the middle phalanx of the right hand. The increases (Δ) in BP from baseline during the first and second minute of the

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CPT were used for analysis. Each reported value reflects the average BP over the final 30 seconds of each minute (i.e., 30–60 seconds and 90–120 seconds of the CPT).

Statistics

Group data are expressed as mean±SD. Baseline characteristics were assessed via independent t-tests or chi-squared analysis for categorical data. BP responses (Δ) to the CPT, as well as plasma NO_3^-/NO_2^- were compared between groups over time using a two-way repeated measures ANOVA. For these comparisons, a power analysis indicated 15 subjects were needed per group for adequate power; a detailed description of this analysis can be found elsewhere.[13] All statistical comparisons were completed using SigmaStat software version 12.0 (Systat Software Inc., San Jose, CA). Significance was set *a priori* at $P<0.05$.

Results

Table 1 illustrates subject characteristics, comorbidities, and drug regimens within the placebo and $NaNO_3$ groups, along with brachial BP prior to supplementation in either group. Eight weeks of $NaNO_3$ supplementation effectively elevated plasma $[NO_3^-]$ and $[NO_2^-]$ compared to placebo, as we have previously reported.[13]

Blood Pressure Responsiveness

Data related to the BP responses to CPT reflect only 11 out of the 13 subjects within the $NaNO_3$ group due to device malfunction and/or poor finger plethysmography recordings. Figure 1 depicts the change (Δ) in systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) during the CPT prior to and following the interventions. $NaNO_3$ ($P<0.01$ and <0.05), but not placebo ($P=0.30$ and 0.72), supplementation reduced ΔSBP during minutes one and two, respectively. No significant group-by-time interactions were observed for ΔDBP ($P=0.72$ and 0.91) or ΔMAP ($P=0.12$ and 0.54) during minutes one or two, respectively.

Discussion

This study examined the effects of eight weeks of $NaNO_3$ supplementation on BP responsiveness in patients with PAD. Previously, we reported improvements in resting brachial SBP, but not brachial DBP or MAP in this same PAD cohort following eight weeks of $NaNO_3$.[13, 14] Within the current study, $NaNO_3$ supplementation improved systolic BP responsiveness to sympatho-excitatory stimuli (CPT) in patients with PAD (Figure 1).

Blood Pressure Responsiveness

Hemodynamic responses to sympatho-excitatory stimuli such as mental arithmetic or CPT are exaggerated in older and hypertensive adults relative to young adults.[9, 10] Exaggerated hemodynamic responses to physiological stressors may predispose aging populations to MACE, and may predict development of hypertension.[6, 7] Recent evidence has demonstrated that an acute dose of NO_3^- (beetroot juice) reduces muscle sympathetic nerve activity (MSNA) in young healthy adults,[15] while enhancing nitric oxide production via L-citrulline reduces the pressor response to a CPT in normotensive men.[16] Within

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the current study eight weeks $NaNO_3$ reduced systolic BP during CPT relative to pre-measurements (Figure 1), with no changes observed in the placebo group. Conversely, $NaNO_3$ supplementation did not have any effect on measures of diastolic BP or MAP relative to placebo. Exaggerated BP responses to physiological stimuli (e.g., exercise, cold stress) are linked to development of hypertension and increase risk for future MACE.[9, 10] Supplementation with $NaNO_3$ reduces exaggerated systolic BP responses to sympatho-excitation in PAD patients. This suggests that $NaNO_3$ supplementation could be a novel way to reduce risk for future MACE, opening this concept for future investigation.

Experimental Considerations

This study was not designed to examine the mechanisms contributing to how BP is reduced with inorganic NO_3^-. Animal models indicate that inorganic NO_3^- (10mmol/L; 14 days in drinking water) attenuates both age-[17] and experimentally-induced hypertension (Angiotensin II + L-NAME)[18] through reductions in NADPH oxidase-mediated oxidative stress, and modulation of angiotensin II receptors within the rostral ventrolateral medulla and kidney, associated with a reduction in sympathetic nerve activity. It could be speculated that the improvements in BP responsiveness to sympatho-excitatory stimuli with $NaNO_3$ supplementation within the current study are perhaps due to these mechanisms, however translation of these mechanistic studies to humans are lacking.

Conclusion

This study sought to examine the effects of eight weeks of supplementation with $NaNO_3$ on BP responsiveness in patients with PAD. Eight weeks of $NaNO_3$ supplementation reduces systolic BP responsiveness to sympatho-excitatory stimuli. Taken together, these results suggest that while $NaNO_3$ supplementation may have positive benefits on BP, these effects are not universally observed across various indices of BP in patients with PAD.

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References

[1]. de Haro Miralles J, Martinez-Aguilar E, Florez A, Varela C, Bleda S, Acin F. Nitric oxide: link between endothelial dysfunction and inflammation in patients with peripheral arterial disease of the lower limbs. Interactive cardiovascular and thoracic surgery. 2009;9:107–12. [PubMed: 19376804]

[2]. Fowkes FG, Aboyans V, Fowkes FJ, McDermott MM, Sampson UK, Criqui MH. Peripheral artery disease: epidemiology and global perspectives. Nature reviews Cardiology. 2017;14:156–70. [PubMed: 27853158]

[3]. Jiang J, Zheng JP, Li Y, Gan Z, Jiang Y, Huang D, et al. Differential contribution of endothelium-derived relaxing factors to vascular reactivity in conduit and resistance arteries from normotensive and hypertensive rats. Clinical and experimental hypertension (New York, NY : 1993). 2016;38:393–8.

[4]. Clement DL, De Buyzere ML, Duprez DA. Hypertension in peripheral arterial disease. Current pharmaceutical design. 2004;10:3615–20. [PubMed: 15579058]

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[5]. Fudim M, Hopley CW, Huang Z, Kavanagh S, Rockhold FW, Baumgartner I, et al. Association of Hypertension and Arterial Blood Pressure on Limb and Cardiovascular Outcomes in Symptomatic Peripheral Artery Disease: The EUCLID Trial. Circulation Cardiovascular quality and outcomes. 2020;13:e006512. [PubMed: 32862697]

[6]. Wood DL, Sheps SG, Elveback LR, Schirger A. Cold pressor test as a predictor of hypertension. Hypertension. 1984;6:301–6. [PubMed: 6735451]

[7]. Schindler TH, Hornig B, Buser PT, Olschewski M, Magosaki N, Pfisterer M, et al. Prognostic value of abnormal vasoreactivity of epicardial coronary arteries to sympathetic stimulation in patients with normal coronary angiograms. Arteriosclerosis, thrombosis, and vascular biology. 2003;23:495–501.

[8]. Schultz MG, Picone DS, Nikolic SB, Williams AD, Sharman JE. Exaggerated blood pressure response to early stages of exercise stress testing and presence of hypertension. Journal of science and medicine in sport. 2016;19:1039–42. [PubMed: 27131837]

[9]. Monahan KD, Feehan RP, Sinoway LI, Gao Z. Contribution of sympathetic activation to coronary vasodilatation during the cold pressor test in healthy men: effect of ageing. The Journal of physiology. 2013;591:2937–47. [PubMed: 23478134]

[10]. Matsukawa T, Gotoh E, Uneda S, Miyajima E, Shionoiri H, Tochikubo O, et al. Augmented sympathetic nerve activity in response to stressors in young borderline hypertensive men. Acta physiologica Scandinavica. 1991;141:157–65. [PubMed: 2048404]

[11]. Ashor AW, Lara J, Siervo M. Medium-term effects of dietary nitrate supplementation on systolic and diastolic blood pressure in adults: a systematic review and meta-analysis. Journal of hypertension. 2017;35:1353–9. [PubMed: 28319596]

[12]. Siervo M, Lara J, Ogbonmwan I, Mathers JC. Inorganic nitrate and beetroot juice supplementation reduces blood pressure in adults: a systematic review and meta-analysis. The Journal of nutrition. 2013;143:818–26. [PubMed: 23596162]

[13]. Bock JM, Treichler DP, Norton SL, Ueda K, Hughes WE, Casey DP. Inorganic nitrate supplementation enhances functional capacity and lower-limb microvascular reactivity in patients with peripheral artery disease. Nitric oxide : biology and chemistry / official journal of the Nitric Oxide Society. 2018;80:45–51.

[14]. Kruse NT, Ueda K, Hughes WE, Casey DP. Eight weeks of nitrate supplementation improves blood flow and reduces the exaggerated pressor response during forearm exercise in peripheral artery disease. American journal of physiology Heart and circulatory physiology. 2018;315:H101–H8. [PubMed: 29522355]

[15]. Notay K, Incognito AV, Millar PJ. Acute beetroot juice supplementation on sympathetic nerve activity: a randomized, double-blind, placebo-controlled proof-of-concept study. American journal of physiology Heart and circulatory physiology. 2017;313:H59–H65. [PubMed: 28476923]

[16]. Figueroa A, Trivino JA, Sanchez-Gonzalez MA, Vicil F. Oral L-citrulline supplementation attenuates blood pressure response to cold pressor test in young men. American journal of hypertension. 2010;23:12–6. [PubMed: 19851298]

[17]. Hezel M, Peleli M, Liu M, Zollbrecht C, Jensen BL, Checa A, et al. Dietary nitrate improves age-related hypertension and metabolic abnormalities in rats via modulation of angiotensin II receptor signaling and inhibition of superoxide generation. Free radical biology & medicine. 2016;99:87–98. [PubMed: 27474450]

[18]. Guimaraes DD, Cruz JC, Carvalho-Galvao A, Zhuge Z, Marques SM, Naves LM, et al. Dietary Nitrate Reduces Blood Pressure in Rats With Angiotensin II-Induced Hypertension via Mechanisms That Involve Reduction of Sympathetic Hyperactivity. Hypertension. 2019;73:839–48. [PubMed: 30712424]

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- PAD is associated with exaggerated blood pressure responses
- Patients with PAD have low nitric oxide (NO) availability
- Inorganic nitrate reduces blood pressure and increases NO in healthy individuals
- 8 weeks of inorganic nitrate in PAD patients reduces BP responses to a cold stress.

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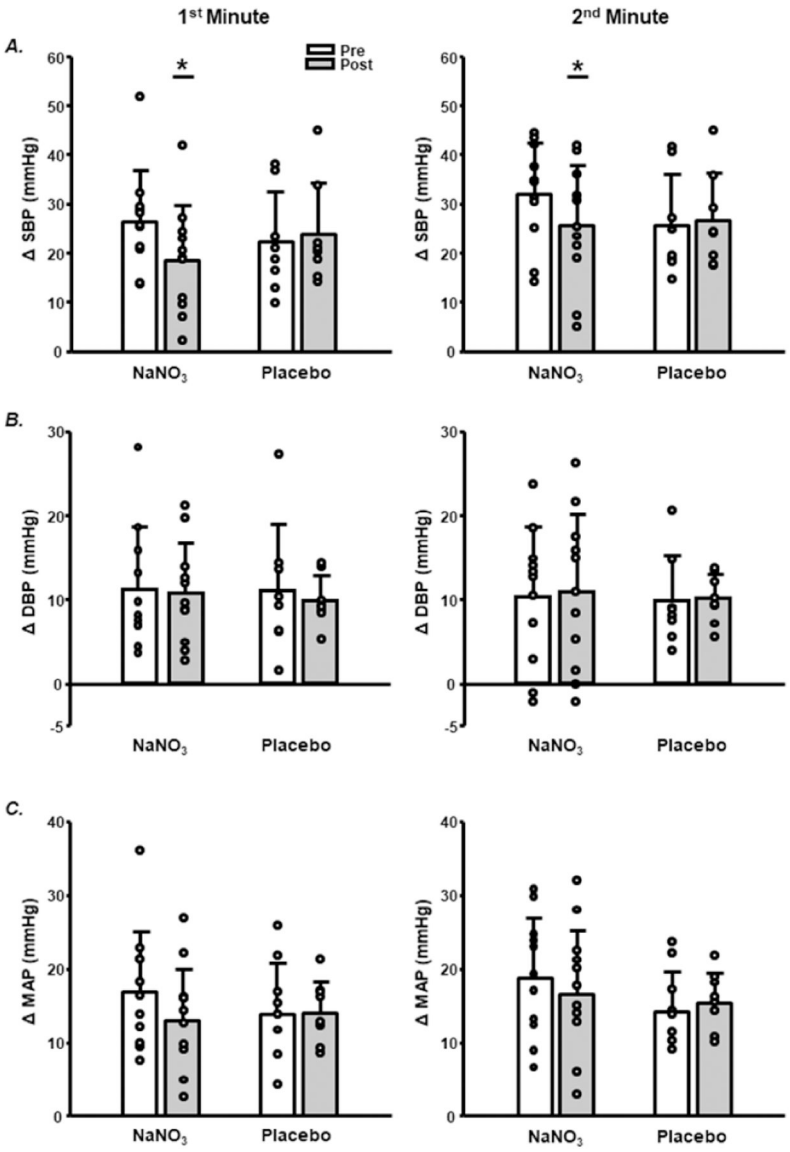

Figure 1:

Change (Δ) from baseline in **A**. systolic blood pressure (SBP), **B.** diastolic blood pressure (DBP), and **C.** mean arterial pressure (MAP) during the first (left column) and last minute (right column) of cold pressor testing pre- and post-supplementation. $NaNO_3$; sodium nitrate. Group data (bars) are mean±SD with circles representing individual responses. *P<0.05 pre-vs. post.

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Table 1:

Subject Characteristics

	NaNO3 (n=13)	Placebo (n=8)	P-value
Age, years	73±9	69±10	0.32
Female, n (%)	7 (54)	2 (25)	0.20
Body mass index, kg/m^2	29.2±5.9	28.1±3.6	0.63
Systolic BP, mmHg	136±15	132±13	0.46
Diastolic BP, mmHg	72±9	77±10	0.30
ABI	0.76±0.21	0.81±0.14	0.57
Medical History, n (%)			
Previous revascularization	12 (92)	6 (75)	0.29
Coronary artery disease	3 (23)	0 (0)	0.15
Type II diabetes mellitus	4 (31)	2 (25)	0.77
Prescription Medication Use, n (%)			
Statin	12 (92)	7 (88)	0.77
ACE inhibitors or ARBs	6 (46)	2 (25)	0.35
Beta-blocker	6 (46)	3 (38)	0.73
Calcium channel blocker	5 (38)	2 (25)	0.55
Anticoagulants	6 (46)	2 (25)	0.35
Insulin	2 (15)	1 (13)	0.90

Mean±SD or n (%). BP, blood pressure; ABI, ankle-brachial index; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker.