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One Week of Daily Dosing with Beetroot Juice Improves Submaximal Endurance and Blood Pressure in Older Patients with Heart Failure and Preserved Ejection Fraction

Joel Eggebeen, MS^{*}, Daniel B. Kim-Shapiro, PhD^{†,‡}, Mark Haykowsky, PhD[§], Timothy M. Morgan, PhD^{||}, Swati Basu, PhD^{†,‡}, Peter Brukaker, PhD^{‡,¶}, Jack Rejeski, PhD^{‡,¶}, and Dalane W. Kitzman, MD^{*,‡}

^{*}Sections on Cardiovascular Medicine and Geriatrics, Wake Forest School of Medicine, Winston-Salem NC

[†]Department of Physics, Wake Forest University, Winston-Salem, NC

[‡]Translational Science Center, Wake Forest University, Winston-Salem, NC

[§]College of Nursing and Health Innovation, University of Texas at Arlington, Arlington, TX

^{||}Department of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, NC

[¶]Health and Exercise Science Department, Wake Forest University, Winston-Salem, NC

Abstract

Objective—To test whether a relatively low single dose or week-long dosing of dietary inorganic nitrate can improve exercise tolerance in patients with Heart Failure with preserved ejection fraction (HFPEF).

Background—Exercise intolerance is the primary manifestation of HFPEF and is largely due to non-cardiac factors that reduce oxygen delivery to active skeletal muscles. A recent study showed improved exercise capacity in patients with HFPEF after a single, acute dose of beetroot juice (BRJ, 12.9 mmol inorganic nitrate) while another recent study showed neutral and negative effects of an organic nitrate.

Address for Correspondence: Dalane W. Kitzman, M, Kermit Glenn Phillips II Chair in Cardiology, Professor of Internal Medicine: Section on Cardiovascular Medicine, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, ; Email: dkitzman@wakehealth.edu, Phone: 336-716-3274; Fax: (336)-716-4995 Or Daniel B. Kim-Shapiro, Harbert Family Distinguished Chair, Professor of Physics, Wake Forest University, Winston-Salem, NC 27109, ; Email: shapiro@wfu.edu, Phone: 336-758-4993, Fax: 336-758-6142

Disclosures: Dr. Kim-Shapiro is listed as a co-inventor on a patent related to use of nitrite in cardiovascular conditions, and owns stock in and serves on the scientific advisory board for Beverage Operations LLC which has licensed Wake Forest University intellectual properties and thus has a financial interest in Beverage Operations LLC. Dr. Kitzman declares the following relationships: Consultant for Abbvie, GSK, Relypsa, Regeneron, Merck, Corvia Medical, and Actavis, grant funding from Novartis, and stock ownership in Gilead Sciences and Relypsa.

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Methods—Twenty HFPEF patients (age: 69 ± 7 years) were enrolled in an initial cross-over design comparing a single, acute dose of BRJ (6.1 mmol nitrate) to a nitrate-depleted, placebo BRJ. A second, one week of daily dosing, phase employed an all-treated design in which patients consumed BRJ for an average of 7 days. The primary outcome of the study was submaximal aerobic endurance, measured as cycling time to exhaustion at 75% of measured maximal power output.

Results—No adverse events were associated with the intervention. Submaximal aerobic endurance improved 24% after one week of daily BRJ dosing ($p=0.02$), but was not affected by the single, acute dose of the BRJ compared to placebo. Consumption of BRJ significantly reduced resting systolic blood pressure and increased plasma nitrate and nitrite in both dosing schemes.

Conclusions—One week of daily dosing with BRJ (6.1 mmol inorganic nitrate) significantly improves submaximal aerobic endurance and blood pressure in elderly HFPEF patients.

Keywords

heart failure with preserved ejection fraction; nitric oxide; exercise; nitrite; nitrate

INTRODUCTION

Heart failure with preserved ejection fraction (HFPEF) is the most common form of HF, is nearly unique to the older population, particularly older women, and is increasing in prevalence (^{1,2}). HFPEF has a distinct pathophysiology compared to heart failure with reduced ejection fraction and thus warrants distinct targeted treatment (³). Exercise intolerance is the primary clinical feature in chronic HFPEF and is a major determinant of these patients' severely reduced quality of life (⁴). Endurance exercise training is currently the only proven therapy to improve aerobic capacity in older HFPEF patients (⁵⁻⁷). Medications tested to date, most of which have primarily targeted cardiac mechanisms, have been unsuccessful (⁸⁻¹⁰).

We have reported that in older HFPEF patients, non-cardiac factors contribute significantly to their exercise intolerance and are the major contributor to its improvement following endurance exercise training (^{7,11,12}). Specifically, skeletal muscle abnormalities, including reduced capillary density, percent type I oxidative fibers, and mitochondrial mass and function coupled with impaired skeletal muscle perfusion may contribute significantly to reduced exercise tolerance in older HFPEF patients (^{5,13-15}). Moreover, it has been suggested that impaired perfusion results are due, at least partially, to low availability of the vasodilator nitric oxide (NO) (¹⁶).

Emerging evidence suggests that dietary inorganic nitrate (NO_3^-) supplementation has beneficial effects on blood pressure control, vascular health, exercise capacity, and oxygen metabolism though targeted NO production (¹⁷⁻¹⁹). It is important to discern inorganic nitrate from organic nitrates such as nitroglycerine and isosorbide mononitrate. The latter was recently shown to decrease daily activity and not effect exercise capacity in patients with HFPEF (²⁰). Both are thought to produce NO or one of its active congeners, but the pharmacokinetics of the two differ considerably. Organic nitrates rapidly release relatively

large amounts of NO, while inorganic nitrate slowly produces NO and thereby produces more mild but sustained vasodilation (21,22). Perhaps most importantly, inorganic nitrate, through its conversion to inorganic nitrite, targets NO delivery to areas of low oxygen and low pH, such as occur in skeletal muscle during exercise (21-23).

Dietary NO₃⁻ is particularly abundant in beetroot juice (BRJ). Indeed, BRJ supplementation has been shown in multiple studies to improve exercise performance and oxygen metabolism in younger healthy individuals (24-31) and in older patients with peripheral arterial disease (32). Specifically, BRJ supplementation has been shown to increase time to exhaustion during high-intensity exercise and to reduce oxygen consumption (VO₂) during submaximal exercise (i.e. reduce oxygen cost at a given submaximal work rate) (25,28,29,32).

In healthy older adults, BRJ supplementation improved VO₂ kinetics but did not alter overall exercise performance or the oxygen cost of exercise (33), and not all studies in younger adults have shown a positive effect (34-36). In addition, some positive effects are seen after one week of daily dosing that are not seen after a single, acute dose (29,31). In a recent study in patients with HFPEF, a single, acute BRJ dose (12.9 mmoles) increased total work performed and cardiac output while decreasing systemic vascular resistance during a maximal exercise test, but did not affect exercise efficiency (as defined by total work/total oxygen consumed) compared to placebo (37). As the optimal BRJ supplementation strategy (single, acute versus loading over several days) remains uncertain,(29) we conducted a pilot study to assess the feasibility (recruitment, retention, safety) and the preliminary efficacy of either a smaller single, acute dose (6.1 mmol) or short-term (1 dose per day of 6.1 mmol for 6-8 days) BRJ supplementation in older HFPEF patients. We hypothesized that one week of daily dosing would be more efficacious than a single dose in improving submaximal aerobic endurance.

METHODS

Participants

As previously described in studies from our laboratory (4,6,11,12,38) and in accord with the ACCF/AHA 2013 HF management guidelines (39), HFPEF was defined as symptoms and signs of HF according to the National Health and Nutrition Examination Survey HF clinical score of 3 and the criteria of Rich et al. (40,41), preserved resting left ventricular systolic function (ejection fraction 50%, and no segmental wall motion abnormalities), and no significant ischemic or valvular heart disease, pulmonary disease, anemia, or other disorder that could explain the patients' symptoms (4,6,11). All patients were required to have significant exercise intolerance (peak VO₂ 16.0 ml/kg/min for women and 20.0 ml/kg/min for men, a reduction of >25% compared to age-matched healthy controls in our laboratory). Further, patients could not be on prescription nitroglycerine, other nitrate preparations used for angina, phosphodiesterase type 5 inhibitors, or medication regimens to alter stomach pH (antacids, proton pump inhibitors, H₂ antagonists). The protocol was approved by the institutional review board, and all participants provided written, informed consent.

BRJ and Placebo

The BRJ and placebo (70 ml; Beet It Sport Shot; James White Drinks, Ipswich, UK) were identical in appearance, taste, smell, and nutrient composition except that the NO_3^- was removed by the manufacturer to make the placebo juice. As measured in our lab, the BRJ contained 0.38 g (6.1 mmol) NO_3^- and the placebo contained 0.0003 g (4.8 mmole) NO_3^- . The person responsible for dispensing the juice was not involved in study testing or analysis.

Study Design

In total, participants completed 5 clinic visits over approximately a 4-week period (Supplementary Figure 1). We initially screened 252 patients from past study participant lists, electronic medical records, and community advertisements. Fifty seven appeared to initially qualify and were scheduled for a screening visit. Of those, 32 signed informed consent and completed the screening visit. Ultimately, 20 participants met all inclusion/exclusion criteria and were enrolled into the study. Following the initial screening visit and a second visit to refine the exercise testing parameters (as described below), participants were assigned using a double-blinded, randomized crossover design to receive BRJ and placebo. A single bottle of the assigned juice was consumed for the crossover visits (single, acute dosing), which were separated by a 3–7 day washout period. Following the last crossover visit, all participants consumed 1 bottle of the BRJ once per day for 6–8 days (one week of daily dosing) and returned for the final clinic visit. Importantly, participants were unaware whether they were consuming BRJ or placebo during the one week of daily dosing period. For all visits, the participant consumed the juice ~ 45 minutes before arriving at the clinic.

Nitrite and Nitrate

After 10 minutes of supine rest (~ 1 hour after the participant consumed the juice), venous blood samples were drawn into 4 ml lithium heparin tubes and centrifuged at 4,000 rpm at 20°C for 3 minutes within 1 minute of collection. Plasma was transferred in 400 μl volumes to sterile 500 μl Sarstedt polypropylene microtubes containing no additives and frozen at -70°C for later analysis. Nitrite (NO_2^-) and nitrate were measured as described previously⁽⁴²⁾ using an ENO-20 nitric oxide analyzer (EICOM, San Diego, CA USA).

Exercise testing

As previously described^(8,38), all exercise tests were performed with the participant in an upright position on an electronically braked cycle ergometer with the pedal rate at ~ 60 rpm. At the initial screening visit, a maximal, graded (10 W per minute) exercise test was performed to assess peak capacity⁽⁴³⁾. The maximal work rate was defined as the greatest work rate that could be maintained for 30 seconds. At all subsequent visits, a submaximal constant work rate exercise test at ~75% of maximal work rate was performed. During all tests, heart rate and rhythm was monitored continuously using an electrocardiogram and blood pressure were taken at rest (following 2 minutes of quiet breathing) and every two minutes during the test. Breath-by-breath gas exchange data (Medgraphics Ultima; Minneapolis, Minnesota) were measured continuously at rest and during exercise. All submaximal constant work rate tests were performed ~ 1.5–2 hours after the participant consumed the juice.

Submaximal aerobic endurance

The submaximal constant work rate exercise test began with a 2-minute period of unloaded pedaling followed by an immediate increase to the specified submaximal work rate, which was maintained until volitional exhaustion. In addition, the test was terminated if the pedal rate fell below 50 rpm for ~ 10 seconds. The submaximal work rate was initially prescribed at 75% of maximal power output. At the second visit (pre-randomization), the adequacy of this work rate was confirmed. If the participant was not able to maintain the work rate for 4 minutes, the work rate was decreased by ~ 10 W for subsequent tests. Likewise, if the participant was able to maintain the work rate for 10 minutes, the work rate was increased by ~ 10 W for subsequent tests. Importantly, the submaximal work rate was the same for all post-randomization efficacy visits.

Study Outcomes

Feasibility was assessed by examining recruitment yield, retention and adherence rates, and safety events. The predetermined primary efficacy outcome was aerobic endurance defined as the exercise time to volitional exhaustion during submaximal cycling at 75% of maximal power output.

Secondary efficacy outcomes included plasma NO_3^- and NO_2^- levels, VO_2 , and blood pressure at rest, after unloaded cycling, at 2 and 4 minutes, and at volitional exhaustion, and heart rate and other gas exchange measures at volitional exhaustion. Values for VO_2 were averaged from the entire resting period and from the final two 15-second averaged values prior to the specified time-point during exercise.

Statistical Methods

Using preliminary data from another study at our institution, the study was designed to have 80% power to detect a 25% difference in the primary efficacy outcome, submaximal exercise time. To determine the single, acute dosing effect of BRJ using the randomized crossover design, comparison of outcome measures were made by repeated measures analysis of variance, controlling for visit. To determine the one week of daily dosing effect of BRJ, comparison of outcome measures were made by a paired t-test, with the placebo visit during the cross-over design as the baseline value and the visit following one week of daily dosing as the final value. A two-tailed p-value of <0.05 was required for significance.

RESULTS

Participants

Twenty older HFPEF patients (age 69 ± 7 years) with typical characteristics of HFPEF were enrolled into the study (Table 1). All participants were New York Heart Association class II (70%) or class III (30%) and 100% had a history of hypertension.

Adherence, Retention, Safety

Adherence to the supplementation, as measured by returned bottle count, was 100%. One participant did not complete the study due to personal time conflicts; therefore 19 participants completed the study. In addition, 1 participant was excluded from the analysis

due to knee pain that resulted in failure to complete the final exercise test. There were no adverse events related to the supplementation.

Plasma NO_3^- and NO_2^-

With single, acute dosing, plasma NO_3^- (362 ± 158 vs. 85 ± 104 μM ; $p < 0.001$) and NO_2^- (0.81 ± 0.91 vs. 0.34 ± 0.26 μM ; $p = 0.01$) were significantly increased. Similarly, with one week of daily dosing, plasma NO_3^- (461 ± 229 vs. 85 ± 104 μM ; $p < 0.001$) and NO_2^- (0.78 ± 0.52 vs. 0.34 ± 0.26 μM ; $p < 0.001$) were significantly increased (Figure 1).

Submaximal aerobic endurance

With single, acute dosing, there was no difference in the primary outcome of submaximal aerobic endurance (BRJ: 352 ± 116 vs. placebo: 377 ± 134 sec.; $p = 0.47$), however with one week of daily dosing, there was a significant increase (449 ± 180 vs. 363 ± 125 sec.; 24% increase; $p = 0.02$) (Figure 2 and Tables 2 and 3).

With both single, acute and one week of daily dosing, there were no differences in VO_2 between BRJ vs placebo at rest or at any time-point measured during exercise, including at volitional exhaustion (Figure 3). Notably, mean respiratory exchange ratio at exhaustion was > 1.15 with all treatments; indicating that although a work rate of $\sim 75\%$ of maximal was used, a similar, exhaustive, severe-intensity level was reached at the end of the test. Finally, with both single, acute and one week of daily dosing, there were no differences in heart rate or any other gas exchange measure at volitional exhaustion (Tables 2 and 3).

Blood Pressure

With single, acute dosing, resting systolic blood pressure was significantly reduced (127 ± 14 vs. 134 ± 14 mmHg; $p = 0.008$) and there was a strong trend to reduced systolic blood pressure after unloaded cycling ($p = 0.06$). Further, there were trends to reduced systolic blood pressure at 2 minutes and volitional exhaustion ($p = 0.15$ for both, Figure 3). There were no differences in diastolic blood pressure or rate pressure product at any time-point.

With one week of daily dosing, systolic blood pressure was significantly reduced at rest (120 ± 13 vs. 134 ± 14 mmHg; $p < 0.001$) and after unloaded cycling ($p = 0.03$). There was a strong trend to reduced systolic blood pressure at volitional exhaustion (159 ± 17 vs. 166 ± 16 mmHg; $p = 0.054$) and there were trends to reduced systolic blood pressure at 2 and 4 minutes ($p = 0.08$ and 0.11 , respectively); Figure 3. Further, there was a strong trend to reduced rate pressure product at rest ($p = 0.051$), with no differences at any other time-point. There were no differences in diastolic blood pressure at any time-point.

DISCUSSION

The primary symptom in patients with chronic HFPEF, even when well compensated, is severe exercise intolerance; this symptom is associated with reduced quality of life⁽⁴⁾. Only endurance exercise training has been shown to improve exercise capacity in these patients, as standard medications tested to date have proven ineffective^(5-8,10). Increased NO bioavailability has been suggested to partially account for the efficacy of exercise training

(16). Our study is the first to examine the safety and efficacy of one week of daily dosing with dietary nitrate in older patients with HFPEF. The major new findings of this study are that: 1) BRJ supplementation was feasible and safe, 2) one week of daily dosing improved submaximal aerobic endurance, and 3) both single, acute and one week of daily dosing reduced resting systolic blood pressure, tended to decrease exercise systolic blood pressure, and increased circulating plasma NO_3^- and NO_2^- levels. These results suggest that one week of daily BRJ supplementation could be a potential therapeutic option to improve submaximal aerobic endurance (as occurs during instrumental and recreational activities of daily living) with a reduction in blood pressure (and thus afterload) in the growing population of older HFPEF patients.

Compared to placebo, either a single, acute dose or one week of daily dosing of BRJ significantly increased plasma NO_2^- similarly, by 138% and 129% compared to placebo, respectively. The blood samples were collected ~ 1 hour after juice consumption and recent evidence suggests that in healthy older adults, plasma NO_2^- levels peak 2–3 hours after BRJ consumption.⁽⁴⁴⁾ Thus, the NO_2^- levels were likely even higher when the exercise test was performed, 1.5–2 hours after BRJ consumption. The increase in plasma NO_2^- is similar to other studies in younger and older adults without HF that have reported a significant increase in plasma NO_2^- following dietary NO_3^- supplementation (24,27,32,33).

One week of daily dosing with BRJ improved submaximal aerobic endurance; no significant effect was found for this outcome with a single, acute BRJ dose. However, a recent study by Zamani and colleagues observed an increase in total work performed after a single, acute dose of BRJ dose in HFPEF patients compared to placebo in a cross-over study (37). These investigators also observed increased cardiac output, decreased systemic vascular resistance and reduced aortic augmentation index due to BRJ compared to placebo, but no improvement in exercise efficiency (total work/total oxygen consumed) was observed (37), a finding confirmed in our study (Figure 3).

It is important to note that our study used a 6.1 mmol dose, whereas Zamani and colleagues administered a 12.9 mmol NO_3^- dose (37). A larger nitrate dose results in increased plasma nitrite (45) which is the essential factor in producing physiological changes. The dose we administered resulted in significantly higher plasma nitrite compared to placebo (Figure 1), but the response was also variable. Comparison of the two studies suggests that a higher nitrate dose may be more effective, at least for acute, single dose effects. The fact that we observed positive effects at the smaller dose we used with chronic but not with the acute dosing schedule is consistent with some previous observations (29,31). That we did not see an improvement in VO_2 suggests that intrinsic mitochondrial function was probably not primarily improved. An increase in perfusion could be dependent on increasing functional capillary density that is achievable after a large single, acute nitrate dose, but needs chronic administration to be achieved with a smaller dose. This is consistent with the notion that microvascular function may be important in oxygen delivery in HFPEF patients (46).

Taken together with the previous study on BRJ and patients with HFPEF (37), the overall data suggest that a single, acute higher dose of BRJ or smaller dose administered over one week can improve aerobic endurance in patients with HFPEF. The mechanism for this effect

is likely due to targeted delivery of NO which decreases systemic vascular resistance. Ingested dietary NO_3^- is reduced to bioactive NO_2^- by bacteria found in the oral cavity; the NO_2^- is then taken up by the plasma from the digestive system and can be converted to nitric oxide (NO), particularly under hypoxic or acidic conditions (23), which can occur in HFPEF and during exercise. In the case of HFPEF, as previously discussed (37,47), improved oxygen cost during exercise is not observed, and increases in exercise capacity are likely due to lower systemic vascular resistance with increased cardiac output (observed in the study published by Zamani and colleagues) (37) and/or more effective shunting of blood flow resulting in increased perfusion of exercising muscles due to the actions of plasma nitrite. Variability in response may be largely due to individual oral nitrate to plasma nitrite conversion which could be improved at larger doses.

A recent study examined the effect of an organic nitrate, isosorbide mononitrate, on improving daily activity level, quality of life score, and 6-minute walk test in 110 patients with HFPEF (20). There were no significant effects of isosorbide mononitrate on quality of life or the 6-minute walk test and activity was actually less compared to placebo (20). Another study examined whether a phosphodiesterase inhibitor, which increases the efficacy of a given amount of NO by increasing levels of its downstream signaling agent cyclic guanosine monophosphate, can improve peak oxygen consumption, exercise capacity and clinical status in patients with HFPEF (48). This study also found no improvement in any of the test-outcomes compared to placebo (48). Differences in the pharmacokinetics and especially the ability of inorganic nitrite derived from inorganic nitrate to target delivery of NO to hypoxic areas (21-23), a feature organic nitrates and oral phosphodiesterase inhibitors lack, is most likely the main reason that positive effects have been seen with use of inorganic nitrate but not these other agents. This notion is also reinforced by a recent study where infused inorganic nitrite improved exercise hemodynamics (particularly pulmonary capillary wedge pressure, cardiac output reserve, and left ventricular stroke work) compared to placebo (49).

Limitations of this study include the small number of participants and lack of mechanistic measures including cardiac output and skeletal muscle perfusion. Another possible limitation would be a learning effect in exercise testing, but previous work in our lab showed no learning effect with maximal exercise testing (38). Further work, with larger sample size, is required to fully evaluate the benefits of oral inorganic nitrate on improving exercise tolerance in patients with HFPEF.

Our study found positive effects of week-long dietary inorganic nitrate intake using a lower dose of nitrate while the previous study by Zamani et al demonstrated positive effects of a single, acute larger dose (37). There are potential benefits to both chronic and acute dosing. For example, if a single, acute dose has an effect, a patient may consume BRJ (probably at a higher dose) a couple of hours before an anticipated activity that may be physically challenging. On the other hand, a patient may chronically consume BRJ (probably at a lower dose) to improve everyday activity and quality of life. However, both studies examining efficacy of inorganic nitrate in treating HFPEF had small sample sizes. Thus, a much larger study should be undertaken examining both acute (single dose) and chronic (over several weeks) dosing of BRJ at several NO_3^- levels.

In summary, our finding that submaximal aerobic endurance was increased at a lower blood pressure suggests that HFPEF patients could potentially perform submaximal exercise at a lower intensity and for longer periods which has implications for everyday activities. Given that a hallmark feature of HFPEF is exercise intolerance even while performing submaximal exercise, our findings may have important functional and therapeutic implications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BRJ	beetroot juice
HFPEF	heart failure with preserved ejection fraction
NO	nitrite oxide
NO₂⁻	inorganic nitrite
NO₃⁻	inorganic nitrate
VO₂	oxygen consumption

References

1. Kitzman DW, Gardin JM, Gottdiener JS, et al. Importance of heart failure with preserved systolic function in patients > or = 65 Years of Age. CHS Research Group. Cardiovascular Health Study. *Am J Cardiol.* 2001; 87:413–419. [PubMed: 11179524]
2. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med.* 2006; 355:251–259. [PubMed: 16855265]
3. Schwartzberg S, Redfield MM, From AM, Sorajja P, Nishimura RA, Borlaug BA. Effects of Vasodilation in Heart Failure With Preserved or Reduced Ejection Fraction: Implications of Distinct Pathophysiologies on Response to Therapy. *J Am Coll Cardiol.* 2012; 59:442–451. [PubMed: 22281246]
4. Kitzman DW, Little WC, Brubaker PH, et al. Pathophysiological characterization of isolated diastolic heart failure in comparison to systolic heart failure. *JAMA.* 2002; 288:2144–2150. [PubMed: 12413374]
5. Haykowsky MJ, Kitzman DW. Exercise physiology in heart failure and preserved ejection fraction. *Heart Failure Clin.* 2014; 10:445–452.
6. Kitzman D, Brubaker P, Morgan T, Stewart K, Little W. Exercise training in older patients with heart failure and preserved ejection fraction. *Circ Heart Fail.* 2010; 3:659–667. [PubMed: 20852060]

7. Kitzman DW, Brubaker PH, Herrington DM, et al. Effect of endurance exercise training on endothelial function and arterial stiffness in older patients with heart failure and preserved ejection fraction: A randomized, controlled, single-blind trial. *J Am Coll Cardiol.* 2013; 62:584–592. [PubMed: 23665370]
8. Kitzman DW, Hundley WG, Brubaker P, Stewart K, Little WC. A randomized, controlled, double-blinded trial of enalapril in older patients with heart failure and preserved ejection fraction; effects on exercise tolerance, and arterial distensibility. *Circ Heart Fail.* 2010; 3:477–485. [PubMed: 20516425]
9. Kitzman D, Upadhyaya B. Heart failure with preserved ejection fraction: a heterogenous disorder with multifactorial pathophysiology. *J Am Coll Cardiol.* 2014; 63:457–459. [PubMed: 24184240]
10. Redfield M, Chen H, Borlaug B, et al. Effect of phosphodiesterase-5 inhibition on exercise capacity and clinical status in heart failure with preserved ejection fraction: A randomized clinical trial. *JAMA.* 2013; 309:1268–1277. [PubMed: 23478662]
11. Haykowsky MJ, Brubaker PH, John JM, Stewart KP, Morgan TM, Kitzman DW. Determinants of Exercise Intolerance in Elderly Heart Failure Patients With Preserved Ejection Fraction. *J Am Coll Cardiol.* 2011; 58:265–274. [PubMed: 21737017]
12. Haykowsky MJ, Brubaker PH, Stewart KP, Morgan TM, Eggebeen J, Kitzman DW. Effect of endurance training on the determinants of peak exercise oxygen consumption in elderly patients with stable compensated heart failure and preserved ejection fraction. *J Am Coll Cardiol.* 2012; 60:120–128. [PubMed: 22766338]
13. Haykowsky M, Brubaker P, Morgan T, Kritchevsky S, Eggebeen J, Kitzman D. Impaired aerobic capacity and physical functional performance in older heart failure patients with preserved ejection fraction: role of lean body mass. *J Gerontol A Biol Sci Med Sci.* 2013; 68:968–975. [PubMed: 23525477]
14. Kitzman DW, Nicklas B, Kraus WE, et al. Skeletal muscle abnormalities and exercise intolerance in older patients with heart failure and preserved ejection fraction. *Am J Physiol Heart Circ Physiol.* 2014; 306:H1364–1370. [PubMed: 24658015]
15. Haykowsky MJ, Kouba EJ, Brubaker PH, Nicklas BJ, Eggebeen J, Kitzman DW. Skeletal Muscle Composition and Its Relation to Exercise Intolerance in Older Patients With Heart Failure and Preserved Ejection Fraction. *Am J Cardiol.* 2014; 113:1211–1216. [PubMed: 24507172]
16. Hirai DM, Musch TI, Poole DC. Exercise training in chronic heart failure: improving skeletal muscle O₂ transport and utilization. *Am J Physiol Heart Circ Physiol.* 2015 Epub ahead of print.
17. Kapil V, Weitzberg E, Lundberg JO, Ahluwalia A. Clinical evidence demonstrating the utility of inorganic nitrate in cardiovascular health. *Nitric Oxide.* 2014; 38:45–57. [PubMed: 24650698]
18. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov.* 2008; 7:156–167. [PubMed: 18167491]
19. Kapil V, Khambata RS, Robertson A, Caulfield MJ, Ahluwalia A. Dietary nitrate provides sustained blood pressure lowering in hypertensive patients: A randomized, Phase 2, double-blind, placebo-controlled study. *Hypertension.* 2015; 65:320–327. [PubMed: 25421976]
20. Redfield MM, Anstrom KJ, Levine JA, et al. Isosorbide Mononitrate in Heart Failure with Preserved Ejection Fraction. *New England Journal of Medicine.* 2015 In Press:null.
21. Lundberg JO, Carlstrom M, Larsen FJ, Weitzberg E. Roles of dietary inorganic nitrate in cardiovascular health and disease. *Cardiovascular Research.* 2011; 89:525–532. [PubMed: 20937740]
22. Omar SA, Artime E, Webb AJ. A comparison of organic and inorganic nitrates/nitrites. *Nitric Oxide-Biol Chem.* 2012; 26:229–240.
23. Cosby K, Partovi KS, Crawford JH, et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nature Med.* 2003; 9:1498–1505. [PubMed: 14595407]
24. Bailey SJ, Fulford J, Vanhatalo A, et al. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J Appl Physiol.* 2010; 109:135–148. [PubMed: 20466802]
25. Lansley K, Winyard P, Fulford J, et al. Dietary nitrate supplementation reduces the O₂ cost of walking and running: a placebo-controlled study. *J Appl Physiol.* 2011; 110:591–600. [PubMed: 21071588]

26. Lansley KE, Winyard PG, Bailey SJ, et al. Acute dietary nitrate supplementation improves cycling time trial performance. *Med Sci Sports Exerc.* 2011;1125–1131. [PubMed: 21471821]
27. Vanhatalo A, Fulford J, Bailey SJ, Blackwell JR, Winyard PG, Jones AM. Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. *The Journal of Physiology.* 2011; 589:5517–5528. [PubMed: 21911616]
28. Bailey SJ, Winyard P, Vanhatalo A, et al. Dietary nitrate supplementation reduces the O₂ cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol.* 2009; 107:1144–1155. [PubMed: 19661447]
29. Hoon MW, Johnson NA, Chapman PG, Burke LM. The effect of nitrate supplementation on exercise performance in healthy individuals: a systematic review and meta-analysis. *Int J Sport Nutr Exerc Metab.* 2014; 23:522–532. [PubMed: 23580439]
30. Cermak N, Gibala MJ, van Loon LJC. Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. *Int J Sport Nutr Exer Metab.* 2012; 22:64–71.
31. Vanhatalo A, Bailey SJ, Blackwell JR, et al. Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology.* 2010; 299:R1121–R1131.
32. Kenjale AA, Ham KL, Stabler T, et al. Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. *J Appl Physiol.* 2011; 110:1582–1591. [PubMed: 21454745]
33. Kelly J, Fulford J, Vanhatalo A, et al. Effects of short-term dietary nitrate supplementation on blood pressure, O₂ uptake kinetics, and muscle and cognitive function in older adults. *Am J Physiol Regul Integr Comp Physiol.* 2013; 304:R73–R83. [PubMed: 23174856]
34. Cermak N, Res P, Stinkens R, Lundberg JO, Gibala MJ, van Loon LJC. No improvement in endurance performance following a single dose of beetroot juice. *Int J Sport Nutr Exer Metab.* 2012 In Press.
35. Glaister M, Pattison J, Muniz-Pumares D, Patterson S, Foley P. Effects of dietary nitrate, caffeine, and their combination on 20 km cycling time-trial performance. *J Strength Cond Res.* 2014; 29:165–174. [PubMed: 24978834]
36. Boorsma R, Whitfield J, Spriet L. Beetroot juice supplementation does not improve performance in elite 1500-m runners. *Med Sci Sport Exerc.* 2014; 46:2326–2334.
37. Zamani P, Rawat D, Shiva-Kumar P, et al. Effect of inorganic nitrate on exercise capacity in heart failure with preserved ejection fraction. *Circulation.* 2015; 131:371–380. [PubMed: 25533966]
38. Scott JM, Haykowsky MJ, Eggebeen J, Morgan TM, Brubaker PH, Kitzman DW. Reliability of peak exercise testing in patients with heart failure with preserved ejection fraction. *Am J Cardiol.* 2012; 110:1809–1813. [PubMed: 22981266]
39. Yancy CW, Jessup M, Bozkurt B, et al. 2013 ACCF/AHA Guideline for the management of heart-failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol.* 2013; 62:e147–e239. [PubMed: 23747642]
40. Rich MW, Beckham V, Wittenberg C, Leven CL, Freedland KE, Carney R. A multidisciplinary intervention to prevent the readmission of elderly patients with congestive heart failure. *N Engl J Med.* 1995; 333:1190–1195. [PubMed: 7565975]
41. Schocken DD, Arrieta MI, Leaverton PE, Ross EA. Prevalence and mortality rate of congestive heart failure in the United States. *J Am Coll Cardiol.* 1992; 20:301–306. [PubMed: 1634664]
42. Berry M, Justus N, Hauser J, et al. Dietary nitrate supplementation improves exercise performance and decreases blood pressure in COPD patients. *Nitric Oxide.* 2015; 48:22–30. [PubMed: 25445634]
43. ACSM. ACSM's Guidelines for Exercise Testing and Prescription. 7. Philadelphia: Lippincott Williams & Wilkins; 2006.
44. Miller GD, Marsh AP, Dove RW, et al. Plasma nitrate and nitrite are increased by a high-nitrate supplement but not by high-nitrate foods in older adults. *Nutrition Research.* 2012; 32:160–168. [PubMed: 22464802]
45. Wylie L, Kelly J, Bailey S, et al. Beetroot juice and exercise: pharmacodynamic and dose-response relationships. *J Appl Physiol.* 2013; 115:325–336. [PubMed: 23640589]

46. Haykowsky M, Tomczak C, Scott J, Patterson D, Kitzman D. Determinants of exercise intolerance in patients with heart failure and reduced or preserved ejection fraction. *J Appl Physiol*. 2015; doi: 10.1152/jappphysiol.00049.2015
47. Vanderpool R, Gladwin MT. Harnessing the nitrate-nitrite-nitric oxide pathway for therapy of heart failure with preserved ejection fraction. *Circulation*. 2015; 131:334–336. [PubMed: 25533965]
48. Redfield MM, Chen HH, Borlaug BA, et al. Effect of Phosphodiesterase-5 Inhibition on Exercise Capacity and Clinical Status in Heart Failure With Preserved Ejection Fraction A Randomized Clinical Trial. *Jama-Journal of the American Medical Association*. 2013; 309:1268–1277.
49. Borlaug BA, Koepp KE, Melenovsky V. Sodium Nitrite Improves Exercise Hemodynamics and Ventricular Performance in Heart Failure With Preserved Ejection Fraction. *Journal of the American College of Cardiology*. 2015; 66:1672–1682. [PubMed: 26449137]

Clinical Perspective

Reduced quality of life in patients with HFPEF is exacerbated by exercise intolerance. Our study suggests that targeted chronic delivery of NO through dietary consumption of oral inorganic nitrate could improve submaximal exercise tolerance. More, larger clinical trials are needed to determine whether dietary inorganic nitrate can truly help improve quality of life in patients with HFPEF.

Translational Outlook

A large variety of clinical trials have recently been completed or are underway exploring the use of inorganic nitrite as a therapeutic for a variety of conditions including hypertension, pulmonary hypertension, myocardial infarction, COPD, PAD and (relevant to the current study) HFPEF. These clinical studies illustrate translation of basic science studies demonstrating how nitrite can be reduced to NO. Whereas some clinical studies employ inhaled or infused nitrite, others (such as this one) deliver nitrite through administration of oral nitrate. The efficacy of this method of administration, which is attractive due to its potential use of natural products and applicability to long-term dosing, needs further exploration.

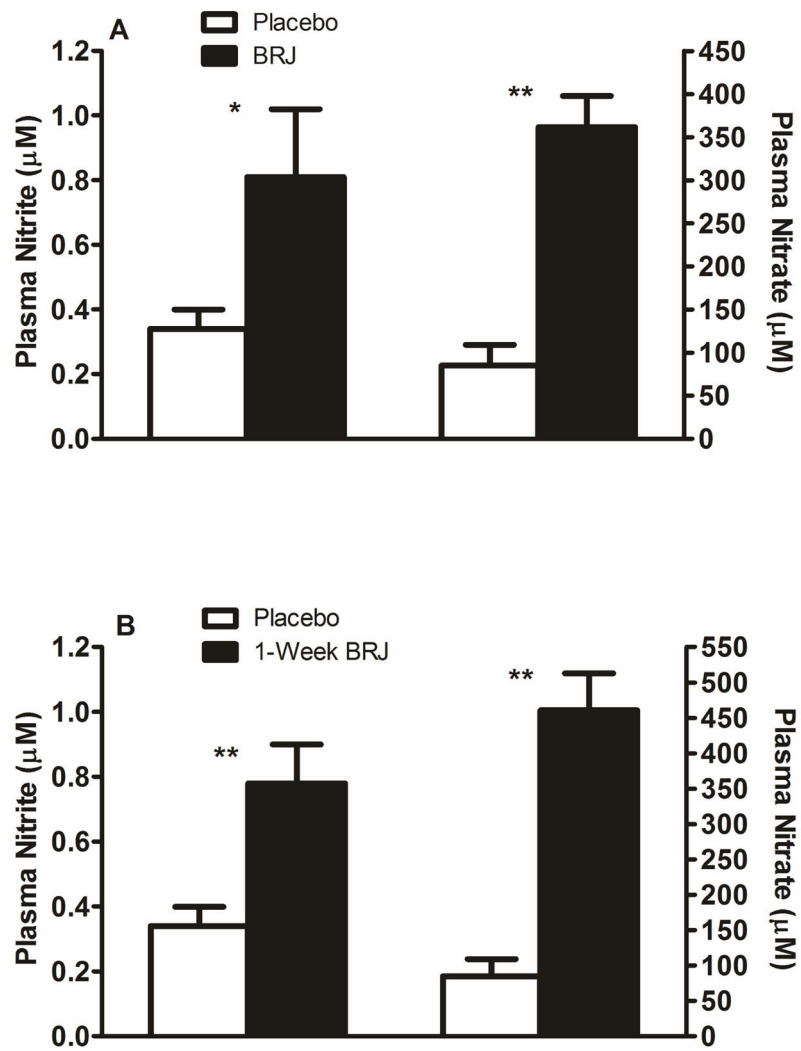


Figure 1. Plasma nitrite and nitrate
 Mean ± SE of plasma nitrite and plasma nitrate after a single, acute dose (panel A) and after 1-week of daily dosing (panel B). White columns represent placebo and black columns represent BRJ. **=p<0.001; *=p<0.05.

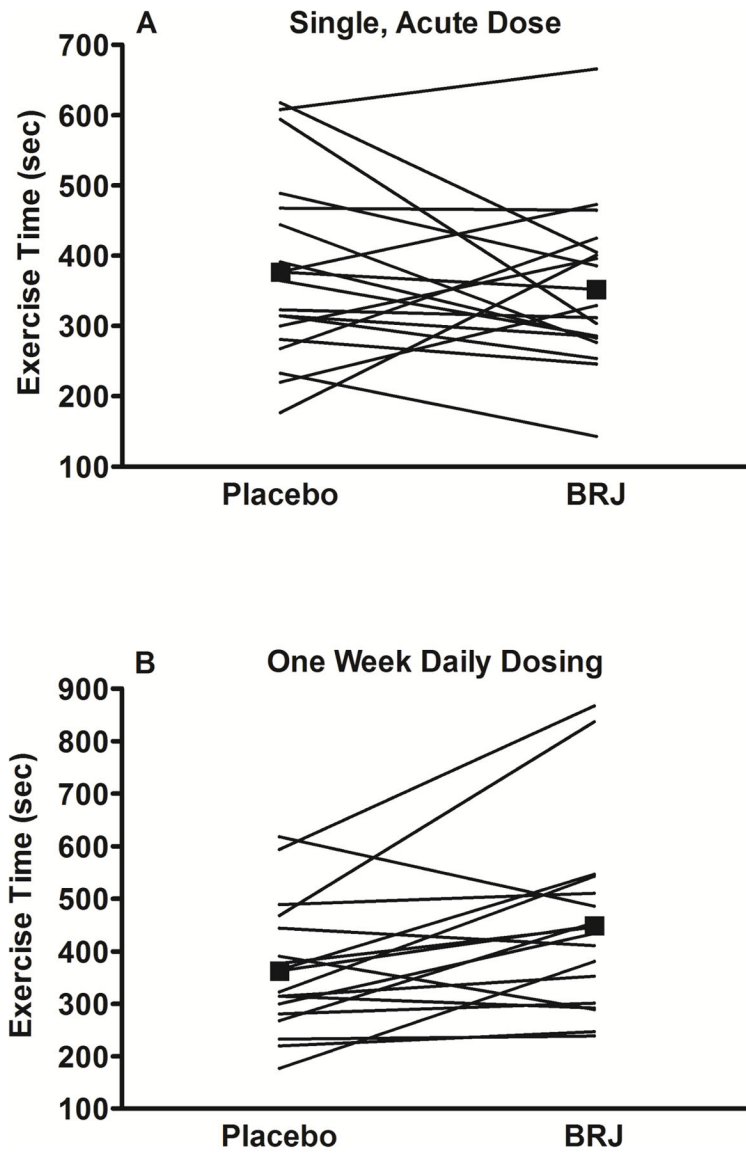


Figure 2. Exercise Time
 Submaximal exercise time (sec.) with placebo and single, acute dose of BRJ (panel A) and with placebo and 1-week of daily dosing of BRJ (panel B). Black squares represent mean values. Circles and lines represent individual values.

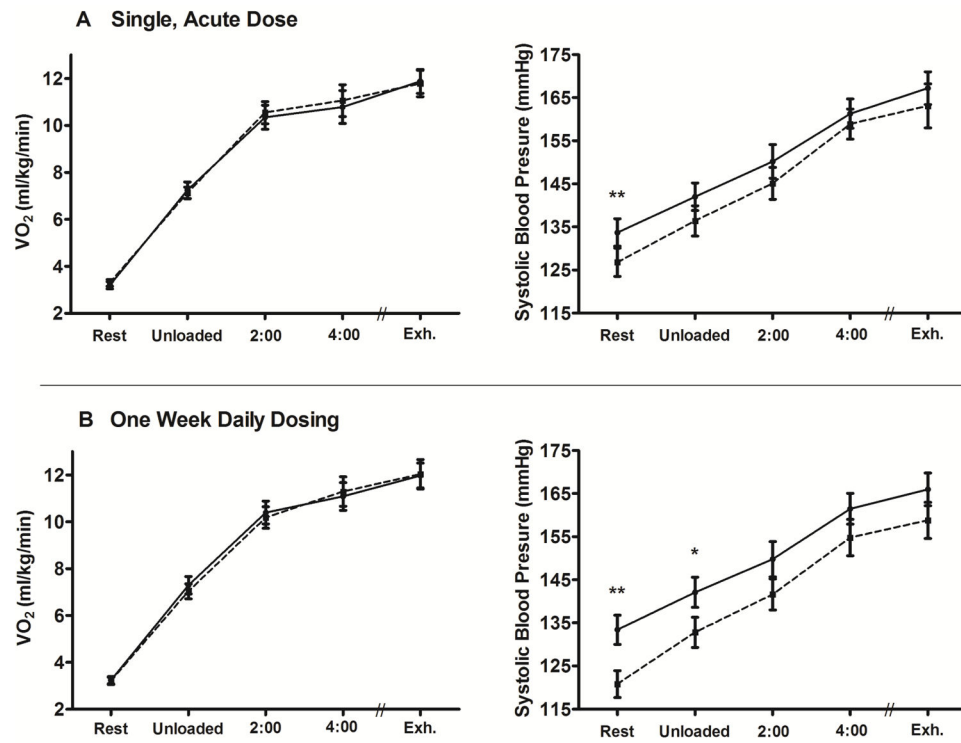


Figure 3. Oxygen Consumption and Blood Pressure
 VO₂ (ml/kg/min) and systolic blood pressure (mmHg) at rest, after unloaded cycling, after 2 and 4 minutes of cycling, and at volitional exhaustion with single, acute dosing (panel A) and with 1-week of daily dosing (panel B). The solid line represents placebo and the dotted line represents BRJ. **=p<0.001; *=p<0.05.

Table 1

Participant Characteristics

Characteristic	HFPEF (N=20)
Age (years)	69.0 ± 6.8
Women	17 (85%)
White	12 (60%)
Body Weight (kg)	89 ± 16
BMI (kg/m ²)	32.9 ± 5.6
NYHA class	
II	14 (70%)
III	6 (30%)
Diastolic Function	
Normal	3 (15%)
Impaired Relaxation	17 (85%)
Pseudonormal	0 (0%)
Restrictive	0 (0%)
e' (cm/s)	6.5 ± 2.0
E/e' ratio	13.2 ± 6.6
BNP*	21 ± 19
Diabetes mellitus	7 (35%)
Hx hypertension	20 (100%)
Systolic BP (mmHg)	145 ± 15
Diastolic BP (mmHg)	70 ± 10
Current medication	
ACE-inhibitors	7 (35%)
Diuretics (all)	13 (65%)
Loop diuretics	3 (15%)
Beta-blockers	5 (25%)
Calcium channel blockers	7 (35%)
ARB's	6 (30%)
Peak VO ₂ (ml/kg/min)	12.0 ± 2.1
Peak workload (watts)	58 ± 21

Data presented as mean ± SD or number (%).

* For BNP median (25th, 75th percentile) was 15 (9, 24).

Abbreviations- BMI: body mass index; NYHA: New York Heart Association; e'; mitral annulus velocity; E; early mitral velocity; BNP, B-type natriuretic peptide; Hx; history; BP: blood pressure; ACE; angiotensin converting enzyme; ARB; angiotensin receptor blocker

Table 2

Acute Effect of Beetroot Juice versus Placebo

	Placebo	Beetroot Juice	P-value
Exercise time at constant work rate, sec	377 ± 134	352 ± 116	0.47
At exhaustion:			
VO ₂ , ml/kg/min	11.9 ± 2.2	11.8 ± 2.4	0.63
VO ₂ , ml/min	1054 ± 315	1040 ± 312	0.43
VCO ₂ , ml/min	1239 ± 418	1211 ± 383	0.28
Respiratory exchange ratio	1.17 ± 0.09	1.16 ± 0.08	0.69
Heart rate, bpm	120 ± 20	121 ± 21	0.69
Systolic blood pressure, mmHg	167 ± 16	163 ± 21	0.15
Diastolic blood pressure, mmHg	78 ± 10	78 ± 10	0.73
Rate pressure product, bpm*mmHg	20245 ± 4491	19997 ± 5009	0.66

Data is presented as mean ± SD. Mean constant work rate = 45 W. Exercise time excludes the 2-minute unloaded pedaling period.

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Table 3

Short term daily dosing effect- 1 dose placebo versus 1 week beetroot juice

	1 Dose Placebo	1 week Beetroot Juice	P-value
Exercise time at constant work rate, sec	363 ± 125	449 ± 180	0.02
At exhaustion:			
VO ₂ , ml/kg/min	12.0 ± 2.2	12.0 ± 2.6	0.79
VO ₂ , ml/min	1065 ± 321	1069 ± 341	0.82
VCO ₂ , ml/min	1259 ± 421	1239 ± 422	0.46
Respiratory exchange ratio	1.18 ± 0.08	1.16 ± 0.09	0.32
Heart rate, bpm	121 ± 21	122 ± 23	0.59
Systolic blood pressure, mmHg	166 ± 16	159 ± 17	0.054
Diastolic blood pressure, mmHg	77 ± 9	75 ± 6	0.38
Rate pressure product, bpm*mmHg	20187 ± 4622	19616 ± 5182	0.43

Data is presented as mean ± SD. Mean constant work rate = 45 W. Exercise time excludes the 2-minute unloaded pedaling period.

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