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Effects of supervised exercise and dietary nitrate in older adults with controlled hypertension and/or heart failure with preserved ejection fraction

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Abstract

Aerobic exercise training is an effective therapy to improve peak aerobic power (peak VO_2) in individuals with hypertension (HTN, AHA/ACC class A) and heart failure patients with preserved ejection fraction (HFpEF). High nitrate containing beetroot juice (BRJ) also improves sub-maximal endurance and decreases blood pressure in both HTN and HFpEF. We hypothesized that combining an aerobic exercise and dietary nitrate intervention would result in additive or even synergistic positive effects on exercise tolerance and blood pressure in HTN or HFpEF. We report results from two pilot studies examining the effects of supervised aerobic exercise combined with dietary nitrate in patients with controlled HTN (n=26, average age 65 ± 5 years) and in patients with HFpEF (n = 20, average age 69 ± 7 years). All patients underwent an aerobic exercise training regimen; half were randomly assigned to consume a high nitrate-containing beet juice beverage (BRJ containing 6.1 mmol nitrate for the HFpEF study consumed three times a week and 8 mmol nitrate for the HTN study consumed daily) while the other half consumed a beet juice beverage with the nitrate removed (placebo). The main result was that there was no added benefit observed for any outcomes when comparing BRJ to placebo in either HTN or HFpEF patients undergoing exercise training ($p \geq 0.14$). There were within-group benefits. In the pilot study in patients with HFpEF, aerobic endurance (primary outcome), defined as the exercise time to volitional exhaustion during submaximal cycling at 75% of maximal power output, improved during exercise training within each group from baseline to end of study, 369 ± 149 sec vs 520 ± 257 sec ($p = 0.04$) for the placebo group and 384 ± 129 sec vs 483 ± 258 sec for the BRJ group ($p = 0.15$). Resting systolic blood pressure in patients with HFpEF also improved during exercise training in both groups, 136 ± 16 mm Hg vs 122 ± 3 mm Hg for the placebo group ($p < 0.05$) and 132 ± 12 mm Hg vs 119 ± 9 mm Hg for the BRJ group ($p < 0.05$). In the HTN pilot study, during a treadmill graded exercise test, peak oxygen consumption (primary outcome) did not change significantly, but time to exhaustion (also a primary outcome) improved in both groups, 504 ± 32 sec vs 601 ± 38 sec ($p < 0.05$) for the placebo group and 690 ± 38 sec vs 772 ± 95 sec for the BRJ group ($p < 0.05$) which was associated with a reduction in supine resting systolic blood pressure in BRJ group. Arterial compliance also improved during aerobic exercise training in both the HFpEF and the HTN patients for both BRJ and placebo groups. Future work is needed to determine if larger nitrate doses would provide an added benefit to supervised aerobic exercise in HTN and HFpEF patients.

Keywords

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

1. INTRODUCTION

The most common form of heart failure is preserved ejection fraction (HFpEF)[1]; it almost exclusively affects older adults and is characterized by exercise intolerance that manifests in a poor quality of life [1–3]. Population studies show that about 90 percent of HFpEF patients have a history of chronic hypertension (HTN). HTN and HFpEF often share common cardiovascular abnormalities including increased arterial stiffness, left ventricular hypertrophy, left atrial dilation, and frequent abnormal diastolic function [4]. Symptoms of both HTN and HFpEF can be improved by aerobic exercise training [5–8]. Blood pressure in treated hypertensives is typically controlled using various drug interventions while aerobic exercise regimens can also reduce blood pressure and improve vascular function [9; 10]. Habitual exercise also improves exercise capacity [10]. To date, the only treatment confirmed in clinical trials to improve exercise capacity in patients with HFpEF is aerobic exercise training [5; 11; 12].

Low nitric oxide (NO) bioavailability results in hypertension and restoration of NO is the basis for the mechanism of action of some current medications [13–15]. Low NO bioavailability has also been suggested to contribute to poor skeletal muscle perfusion in patients with HFpEF [16], which (along with other non-cardiac factors) contributes to exercise intolerance [11; 17–19]. One attractive means to deliver NO is through the anion nitrite (NO_2^-) as nitrite is reduced to NO preferentially in areas of low oxygen and pH so that delivery is well-targeted to metabolically active tissue such as at the muscles involved in exercise [20]. This is often accomplished through the nitrate (NO_3^-)-nitrite-NO pathway [21; 22]. Plasma nitrate is derived from endogenous mechanisms (including the oxidation of NO) and from dietary consumption (especially vegetables including beets and beet root juice [23]). Bacteria in the oral cavity partially reduce salivary nitrate to nitrite [24]. Nitrate and nitrite in the gastrointestinal tract are transferred to the plasma. Nitrite is then reduced to NO, preferentially under hypoxic and acidic conditions, through mechanisms proposed to involve a variety of heme and non-heme proteins, as recently reviewed [25]. Plasma nitrate is concentrated in salivary glands and secreted back into the oral cavity so that the nitrate-nitrite-NO pathway cycles for an extended period with the half-life of plasma nitrate being about six hours [21; 26; 27].

Several studies have demonstrated the therapeutic potential of the nitrate-nitrite-NO pathway including that dietary nitrate lowers blood pressure and improves exercise performance in patients with chronic obstructive pulmonary disease [28], improves exercise performance in patients with peripheral artery disease [29], and improves exercise capacity and endurance in

[1]Abbreviations: heart failure is preserved ejection fraction (HFpEF); hypertension (HTN); exercise (EX); beet root juice (BRJ); peak volume oxygen consumption ($VO_{2\ peak}$); stroke volume (SV); cardiac output (CO); systemic vascular resistance (SVR); total arterial compliance (TAC); velocity index (VI); acceleration index (AI); left cardiac work index (LCWI); impedance cardiography (ICG); systolic blood pressure (SBP); diastolic blood pressure (DBP); heart rate variability (HRV); blood pressure variability (BPV); and baroreflex sensitivity (BRS); low frequency (LF); and high frequency (HF); standard deviation of normal beat-to-beat interval (SDRR); root of mean of successive differences (rMSSD); Blood pressure variability (BPV); standard deviation of the mean arterial pressure (SDMAP); best-responder (BR), good-responder (GR); non-responder (NR); analysis of variance (ANOVA); body mass index (BMI); angiotensin converting enzyme (ACE); angiotensin receptor blocker (ARB); New York Heart Association (NYHA); mitral annulus velocity (e'); early mitral velocity (E); history (Hx); respiratory exchange ratio (RER); heart rate (HR); minute ventilation (VE); ventilatory anaerobic threshold (VAT); ambulatory blood pressure measurements (APBM); mean blood pressure (MBP); Power of spectrum in high frequency range (Hfa); slope of the baroreflex gain curve measured by the sequence method in the UP or Down direction (Seq UP & DOWN).

patients with HFpEF [30; 31]. Numerous studies have shown that dietary nitrate improves exercise efficiency or performance, or lowers blood pressure in healthy volunteers [32]. A recent double-blind, placebo controlled study demonstrated sustained blood pressure lowering due to dietary nitrate in hypertensive individuals that were both taking medication and drug-naïve [33]. In addition, it has recently been shown that infused [34] or inhaled [35] nitrite (the active metabolite of nitrate) improves hemodynamics in patients with HFpEF.

We hypothesized that the combination of oral nitrate and aerobic exercise training will improve nitric oxide bioavailability as well as blood pressure and result in improved exercise performance beyond what is observed with aerobic exercise training alone. This hypothesis is based on the notion that the effect of aerobic exercise training may be limited by poor NO bioavailability due to endothelial dysfunction. Simultaneous administration of oral nitrate could improve aerobic exercise training and subsequent outcomes. This hypothesis is supported by recent work showing that dietary nitrate results in similar physiological responses as exercise therapy in a diabetic rat model [36]. We tested our hypothesis in two separate studies: patients with HFpEF and individuals with controlled hypertension.

2. METHODS

2.1 Study Design

Both studies (HFpEF and HTN) were approved by the institutional review board, and all participants provided written, informed consent.

HFpEF Study—This pilot study was an extension of a recent study of 20 HFpEF patients (average age 69 ± 7 years) investigating dietary nitrate (but no exercise) [31]. Briefly, the recent published study examined effects of a single acute dose and one week of daily dosing of BRJ (with no exercise). This portion of the study that did not involve exercise training lasted between 12 and 22 days from the end of baseline exercise testing and the last visit of that previous study. Immediately following the final study visit of the previous report [31], all participants were entered into a 4-week exercise program and were randomized to receive either beetroot juice (BRJ, 70 ml; Beet It Sport Shot; James White Drinks, Ipswich, UK) or placebo to consume before each exercise session (Figure 1). The BRJ and placebo were identical in appearance, taste, smell, and nutrient composition except that the NO_3^- was removed by the manufacturer to make the placebo. 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 μmole) NO_3^-. The person responsible for dispensing the juice was not involved in study testing or data analysis.

Participant inclusion and exclusion criteria, recruitment, and enrollment were reported previously [31]. Participants' characteristics are summarized in Table 1. Following the intervention, participants completed two clinic visits separated by 1–4 days. Baseline values were considered to be from the placebo crossover visit from our previous report [31]. For all visits, the participant consumed the assigned juice ~45 minutes before arriving at the clinic so that they would begin aerobic exercise at one hour after consumption.

All participants completed a medical facility-based, supervised, 4-week exercise program. There were three exercise sessions per week held on Mondays, Wednesdays, and Fridays. The sessions consisted of a series of warm-up, flexibility, and aerobic exercises, carried out with a combination of walking (circuit and motorized treadmill with adjusted speed and grade) and cycling (Airdyne and/or recumbent bike) exercise. Participants spent approximately half of the aerobic exercise time in each mode of exercise training. The goal was to build up to at least 40 minutes of training (20 minutes walking and 20 minutes cycling) by the end of the 4-week program. The intensity started at a low to moderate intensity level (50–60% of heart rate reserve from baseline maximal exercise test) and gradually increased until the participant was able to maintain 70% of heart rate reserve for at least 20 minutes. During the cycling portion, participants exercised at 40–60% of peak workload (from the baseline maximal exercise test, measured as watts).

Subjects were asked to refrain from using mouthwash on each testing day and for 2 days prior to any scheduled consumption of the beetroot juice or placebo. Participants were instructed to consume the BRJ or placebo ~1 hour before arriving at the exercise facility along with a breakfast meal. Thus, we refer to groups as exercise + BRJ (EX + BRJ) or EX + placebo. A log of the exact time of ingestion was completed and verbal confirmation of juice consumption was obtained before each exercise session. Importantly, the BRJ or placebo was only consumed on exercise days.

HTN Study—A randomized, placebo-controlled double blind trial was performed where participants (n=26, average age 65 ± 5 years) with controlled hypertension were assigned to aerobic exercise with either nitrate rich beet root juice (BRJ, n=13, 6F) or nitrate-depleted beetroot juice placebo drink (Placebo, n=13, 7F). The BRJ (Beet It Sport Shot; James White Drinks, Ipswich, UK) for the HTN pilot study contained 0.5 g (8 mmol) NO_3^- and the placebo contained 0.001 g (20 μmole) NO_3^-. Participants were aged ≥55 years, had a systolic blood pressure between 130–160 mm Hg, were taking no more than 2 hypertensive medications, and were sedentary, defined as performing <60 minutes of moderate levels of exercise each week performed in bouts >10 minutes. Exclusion criteria included the use of tobacco products, involvement in another research study, and a Modified Mini-Mental State Exam (MMMSE) score <80. Furthermore, participants were ineligible if they were taking medications known to interfere with nitrate/nitrite metabolism, diagnosed with active neurological dysfunction, or had contraindications for participation in exercise. Patient characteristics are summarized in Table 2. The aerobic exercise intervention consisted of a center-based, individualized, moderately intense walking program of 18 sessions: three 50-minute sessions per week for 6 weeks. All participants walked on motorized Life Fitness TR-9500HR treadmills at a Borg rating of perceived exertion of 12–13. The first four sessions were used to acclimatize participants to the training protocol and progressively increase the walking time towards the goal of 50 min per session. Participants were guided through a series of stretching exercises before and after walking. The participants were instructed to consume one 70 mL (2.4 oz.) beverage each day of the week within 30 minutes of opening the bottle. On days that they were scheduled to attend the exercise intervention they were to consume the beverage 1 hour before the scheduled training session time. Daily consumption logs were completed by all participants and collected weekly. Volunteers were

asked to avoid antacids, spinach, lettuce, beets, and leafy greens for the duration of the study. They were given these guidelines on a business card. We did not formally track compliance with the requested dietary restrictions. Height was measured to the nearest 0.5 cm using a stadiometer and body mass was measured to the nearest 0.1 kg using a scale. BMI (kg/m^2) was calculated as body mass divided by height squared.

2.2 Nitrite and Nitrate

Venous blood samples were drawn into BD 4 ml lithium heparin tubes and centrifuged at 4,000 rpm at room temperature for 3 minutes within 1 minute of collection. For the HTN study, blood was drawn immediately before and one hour after BRJ or placebo consumption. For the HFpEF study, blood was drawn one hour after BRJ or placebo consumption. Plasma was transferred in polypropylene microtubes containing no additives and frozen at −70°C for later analysis. Nitrite and nitrate were measured as described previously [37] using an ENO-20 nitric oxide analyzer (EICOM, San Diego, CA USA). Plasma was mixed with equal volume of 100% methanol, vortexed and centrifuged at 11,500g for 10 minutes. The supernatant was loaded into 96 well plates and nitrate and nitrate concentrations was measured using ENO-20 NOx analyzer (EICOM, San Diego, CA, USA). The nitrite and nitrate is separated via column chromatography and reacted individually with the Greiss reagent to synthesize a red diazo compound that is read at a wavelength of 540 nm by a visible light detector in the ENO-20, sample preparation described above are as per the manufacturer's instructions. Standard nitrite and nitrate samples were made freshly each day before plasma samples were measured and used for calibration.

2.3 Exercise testing

HFpEF Study—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, as previously described [31; 38; 39]. Two exercise tests, conducted on separate visits, were completed before and after the intervention. The first exercise test was a maximal, graded (10 W per minute) exercise test performed to assess peak aerobic capacity [40]. The maximal work rate was defined as the greatest work rate that could be maintained for ≥ 30 seconds. The second exercise test was a submaximal constant work rate exercise test at ~75% of maximal work rate performed to assess submaximal aerobic endurance. The details of the submaximal constant work rate test were described previously [31]. Breath-by-breath gas exchange data (Medgraphics Ultima; Minneapolis, Minnesota) were measured continuously at rest and during exercise. All tests were performed ~ 1.5–2 hours after the participant consumed the juice. (BRJ or placebo).

HTN Study—We used a physician supervised; individualized ramp treadmill protocol, where subjects walked at a brisk pace with the speed based on their comfort and fitness. The grade was increased at a rate of 1–2 %/minute based on fitness level. Peak volume oxygen consumption ($VO_{2\ peak}$) was determined during the graded exercise test using a metabolic cart (Medical Graphics Ultima). Heart rate, rhythm, blood pressure, and oxygen uptake were monitored during the test. Test termination criteria include: patient request, volitional fatigue, increasing chest or leg pain, dizziness, faintness, fatigue, pallor, cyanosis, ataxic gait, cardiac arrhythmias or decompensation, hyper or hypotension, and ECG abnormalities.

2.4 Hemodynamic Measures

HFpEF Study—During all tests, heart rate and rhythm were monitored continuously using an electrocardiogram, and blood pressure was taken at rest (following 2 minutes of quiet breathing) and every two minutes during the test.

Doppler-echocardiograms were performed and analyzed according to American Society of Echocardiography recommendations and as previously described with a Phillips iE33 machine and Xcelera work station (Phillips Medical Systems, The Netherlands) [5; 12; 38; 41–44]. Doppler LV filling patterns [45; 46] and mitral annulus tissue velocities (septal) were assessed as previously described [41; 47].

HTN Study—Ambulatory 24-hour blood pressure and heart rate: Subjects were asked to wear blood pressure monitors (Spacelabs 90207, Redmond, WA) for 24 hours and keep a diary of their activities. The monitors were fitted by a trained researcher and programmed to inflate every 30 minutes from 6 am to 11 pm and every 60 minutes from 11 pm to 6 am. Subjects with at least 80% valid readings were analyzed (n=12 placebo and n=12 BRJ). Mean SBP and DBP of daytime and night-time were calculated according to self-reported sleep and awake times. The percentages of nocturnal SBP and DBP decline were determined using the following formula: [(daytime BP-night-time BP)/daytime BP] ×100. Inadequate nocturnal dipping was defined as less than 10% reduction in the mean SBP from awake to asleep [48; 49].

Measures of stroke volume (SV), cardiac output (CO), systemic vascular resistance (SVR), total arterial compliance (TAC), velocity index (VI), acceleration index (AI) and left cardiac work index (LCWI) were derived from mean arterial pressure measures obtained using impedance cardiography (ICG) machine (BioZ®, Model BZ 4110-101D) [50; 51]. This machine applies Ohm’s relationship to the thorax to allow changes in voltage and impedance due to changes in blood flow to be translated into hemodynamic parameters of cardiovascular function [52; 53].

Continuous BP and HR were acquired from noninvasive finger arterial pressure measurements for a minimum of 5 minutes in subjects in the supine position, at baseline and followup. SBP and RR intervals (RRI) files were acquired (BIOPAC acquisition software, Santa Barbara, CA) at 1000 HZ and analyzed using Nevrokard BRS software (Nevrokard BRS, Medistar, Ljubljana, Slovenia) followed by post-hoc analysis for autonomic function variables (heart rate variability (HRV), blood pressure variability (BPV) and baroreflex sensitivity (BRS)). BRS was measured using a frequency domain method as LF, HF which is the square root of the spectral density of the heart rate divided by the square root of the spectral density of systolic arterial pressure in the low frequency (LF), and high frequency (HF) range, and using a time domain method as Seq UP & DOWN (slope of the baroreflex gain curve measured by the sequence method in the UP or Down direction; Seq ALL: slope of all the baroreflex curves), while HRV was measured as power of RRI spectra in LF, HF range and their ratio (LF_{RRI}/HF_{RRI}) and standard deviation of normal beat-to-beat interval (SDRR) and root of mean of successive differences (rMSSD). Blood pressure variability (BPV) was measured as standard deviation of the mean arterial pressure (SDMAP) as described previously [54–56].

2.5 Study Outcomes

HFpEF Study—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, peak VO_2 from the maximal exercise test, resting Echo-Doppler characteristics, 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 of the submaximal constant work rate exercise test. VO_2 outcomes were defined as the average of the entire resting period and the average of the 30 seconds prior to each specified timepoint (i.e. 2 minutes, 4 minutes, peak)

HTN Study—The primary efficacy outcome was exercise capacity (assessed by peak oxygen consumption and time to volitional exhaustion) during a graded treadmill exercise test. Secondary outcome measures included blood pressure, autonomic and vascular function including vascular resistance, and heart rate variability (at rest or 24 hr monitoring or during the exercise test).

2.6 Ad hoc analysis

For the HTN study, the degree to which individuals converted oral nitrate to plasma nitrite was compared to improvement in exercise time. The change in nitrite was taken from levels measured before and after BRJ consumption. The groups were best-responder (BR, change greater than 150 nM), good-responder (GR, change less than 150 nM but greater than 50 nM) and non-responder (NR, change less than 50 nM). These groups had n=4, n=4, and n = 3 individuals each respectively.

2.7 Statistical Methods

Based on preliminary data from a study of chronic obstructive pulmonary disease patients, this randomized pilot study of HFpEF patients was designed to have 80% power to detect a 40% difference in the primary outcome. The HTN study did not include any a priori power calculations as it was considered a pilot study. Comparisons of participant characteristics were made by Student’s t-test for continuous variables or by Fischer’s exact test for categorical variables. For participant characteristics, NYHA class and diastolic function, in Table 2, an alternative comparison of outcome measures between the groups was made by a two way analysis of variance (ANOVA) followed up with a Tukey's characteristic-wise test. Comparison of outcome measures between intervention groups were made by analysis of covariance, with the follow-up value adjusted for the baseline value using the least square mean. A two-tailed p-value of <0.05 was required for significance. Paired samples t-tests were performed for comparison within groups (pre vs post) with a p-value <0.05 required for significance.

3. RESULTS

3.1 Retention, Adherence, Safety

HFpEF Study—All participants completed the intervention and follow-up testing. Participants attended an average of 90% of the exercise sessions. Adherence to the BRJ

supplement, as measured by returned bottle count and consumption log, was 100%. There were no adverse events related to the aerobic exercise training program or BRJ supplement.

HTN Study—Adherence to the exercise intervention was 85% with 22 out of 26 volunteers having 100% adherence. Daily logs were used to assess adherence to the supplement. Only three of the 26 participants were not 100% adherent with one volunteer in the placebo group being 86% adherent in taking the supplement and two volunteers in the BRJ group being 98% adherent. There were 2 adverse events in the study, both of which were related to the exercise intervention (both participants had a fall while walking on the treadmill). One participant sustained a severe muscle strain and withdrew from the study and one participant sustained minor bruising and returned to training at the next exercise session.

3.2 Plasma NO_3^- and NO_2^-

HTN Study—EX + BRJ resulted in both elevated nitrate and nitrite compared to EX + placebo: 422 ± 127 μM nitrate for BRJ and 21 ± 8 μM nitrate for EX + placebo, and 0.32 ± 0.18 μM nitrite for BRJ and 0.14 ± 0.07 μM nitrite for EX + placebo, $p<0.001$ (Figure 2). Plasma nitrite and nitrate was measured at weeks 1, 2, and 3 before and after beverage consumption. Interestingly, average plasma nitrate in volunteers taking BRJ increased from baseline at week 1 (32 μM before BRJ and 344 μM after consumption) to week 2 (114 μM before BRJ and 459 after consumption, p = 0.002) and again (compared to week 1) in week 3 (108 μM before BRJ and 467 μM after consumption, p = 0.0002). However, no changes in nitrite levels between weeks was noted nor was the change in nitrate (post-pre consumption) different at weeks 1, 2, or 3. Oddly, plasma nitrite after placebo consumption was lower in week 3 compared to week 1 (p = 0.04). No other differences in these measures were noted between weeks.

HFpEF Study—Plasma NO_3^- was significantly ($p<0.001$) elevated in the BRJ group (EX + BRJ: 327 ± 30 μM) compared to the EX + placebo group (EX: 70 ± 31 μM). In contrast, plasma NO_2^- (BRJ: 0.73 ± 0.16, Placebo: 0.47 ± 0.17 μM; p=0.27) was not different between the groups (Figure 2).

Notably, plasma NO_2^- and NO_3^- were higher in the placebo group from the HFpEF group compared to the HTN group. Part of the reason that the plasma nitrite placebo value is so high in the HFpEF study is due to a single individual whose plasma nitrite concentration was measured to be 3.15 μM. When this person's measure is excluded, the average, adjusted plasma nitrite levels for the placebo measure becomes 0.27 ± 0.17 μM (from 0.46 ± 0.17 μM when that person is included). Similarly, this same person had an extremely high level of plasma nitrate (332 μM) and excluding that value brings the average adjusted plasma nitrate values down from 70 ± 31 μM to 58 ± 34 μM. Exclusion of this single measure still did not result in a significant elevation in plasma nitrite in comparing the placebo and BRJ groups for the HFpEF study

3.3 Exercise testing

HFpEF Study—There was no significant difference in submaximal aerobic endurance (the primary outcome) between the BRJ and placebo groups (BRJ: 475 ± 60 vs. placebo: 531

± 68 sec.; p=0.55). The expected exercise-induced improvement in submaximal aerobic endurance was observed in both populations (26% improvement in the BRJ group and 41% improvement in the placebo group, p<0.05 for placebo group, p=0.01 when groups combined) (Table 3).

In the BRJ group there was a trend for VO_2 to be lower at volitional exhaustion compared to EX + placebo (p=0.14). There were no other differences in VO_2 between the BRJ and placebo groups at rest or at any time-point measured during exercise (Figure 3A). Mean respiratory exchange ratio at exhaustion was ≥ 1.12 in both groups and at both time points; indicating that although a work rate of ~75% of maximal was used, a similar, exhaustive, severe-intensity level was reached at the end of the test (as indicated by the similar respiratory exchange ratios which were not significantly different from each other). Finally, there were no differences in heart rate or any other gas exchange measure at volitional exhaustion (Table 3).

There was no difference in peak VO_2 or any other maximal exercise gas exchange measure, ventilatory anaerobic threshold, or VE/VCO_2 slope comparing the BRJ and placebo groups (Table 4). Both groups improved exercise time; the change was significant in the placebo group (Table 4).

HTN Study—Following six weeks of self-paced treadmill walking, there was no added benefit of consuming BRJ compared to placebo when combined with exercise training for VO_2peak (p>0.74). Both populations did exhibit significant increases in time to exhaustion and trends for increased peak VO_2 (Fig 4A and B, Table 5).

In ad hoc analyses, we compared changes in nitrite levels to the change in exercise time. As described in the methods section, participants were broken into three groups, best responders, good-responders, and non-responders based on the increases observed in plasma nitrite following BRJ consumption. The improvement in exercise time trended to be greatest for best-responders and least for non-responders (Figure 5).

3.4 Hemodynamic Measures

HFpEF Study—With BRJ, there was a trend to lower systolic blood pressure after unloaded pedaling (p=0.16) and a trend to lower diastolic blood pressure at volitional exhaustion (p=0.15) compared to placebo. There were no group differences in systolic or diastolic blood pressure at rest or at any other time point during the submaximal constant work rate exercise test (Table 3, Figure 3B). Resting systolic blood pressure was reduced following the intervention in both the BRJ (9%, p=0.007) and placebo (10%, p=0.04) groups, indicating that BRJ had no added benefit to exercise (Table 3).

There was no difference between the groups in any Echo-Doppler measures (Table 6).

HTN Study—For 24-h ambulatory blood pressure, 12 subjects in each group had >80% of the recordings valid. Blood pressure measurements at baseline and after 6 weeks of exercise training intervention in both groups are presented in Table 5. Blood pressure (systolic,

diastolic and mean) and heart rate were not significantly different after exercise intervention in both groups.

Supine resting systolic blood pressure decreased significantly in the BRJ group but not in the Placebo group (Table 5, Figure 4C). The between group comparison was not significant (p=0.22), suggesting no significant added benefit of BRJ. Changes in supine resting diastolic blood pressure were not significant (Table 5, Figure 4D).

Both study groups experienced significantly improved arterial compliance compared to baseline (Table 5) and a trend towards a reduction in LCWI in BRJ group (p=0.07) (Table 5). There were no significant changes in any of the other recorded hemodynamic measures in either group (Table 5).

There was a 15–20% increase in measures of baroreflex sensitivity in both groups with no group differences (Table 1- supplement). There was also a 10–20% reduction in blood pressure variability measured as SDMAP and a 15–30% reduction in the LF/HF sympathovagal index. None of these changes reached statistical significance and there were no between group differences (p>0.21).

4. DISCUSSION

Previous work has shown that either exercise alone or dietary nitrate alone can decrease blood pressure in hypertensives and improve exercise tolerance in patients with HFpEF [5; 9–12; 30; 31; 33; 57; 58]. In addition, dietary nitrate has been shown to increase exercise efficiency, tolerance, and performance in various other populations [28; 29; 59–64]. We reasoned that an increase in perfusion due to increased NO bioavailability from dietary nitrate (as demonstrated in the case of exercising muscle in rats [65]) would allow patients to exercise at longer or higher levels of an exercise stimuli and potentially achieve a greater benefit from exercise training. We thus hypothesized that combining an exercise regimen and a dietary nitrate intervention would have an additive, if not synergistic, effect on outcomes in patients with hypertension and HFpEF. In general, the two studies presented here do not support this hypothesis; dietary nitrate appeared to have no significant added benefit compared to placebo when combined with an aerobic exercise training regimen.

In the HFpEF pilot study, the primary efficacy outcome, aerobic endurance capacity, improved due to the exercise regimen within both groups, but there was no significant added benefit of adding BRJ (p = 0.55). In fact, although there was a trend for improvement in the BRJ group, only the change observed in the placebo group reached significance. In the HTN pilot study, the primary efficacy outcome, exercise capacity, trended to improve within each group, but the trend for improvement was not greater in the BRJ group than the placebo group (p = 0.98, Figure 4A, Table 5). Exercise time increased significantly within both groups, but the improvement in exercise time for the BRJ group was not greater than that of the placebo group (p = 0.74, Figure 4B, Table 5).

Similar results were observed in secondary efficacy outcomes. In the HFpEF study, resting systolic blood pressure was significantly lowered in both groups, but there was no added benefit of BRJ (Table 3). In the HTN study there were no changes in blood pressure

compared to baseline, but both total arterial compliance improved within both groups with no added benefit of BRJ (Table 5). No other significant changes were observed in any other outcome measures.

Whereas we observed no added benefit of BRJ consumption when combined with exercise, many previous studies have demonstrated benefits of BRJ consumed in the absence of a simultaneous exercise regimen. Several studies have shown that dietary nitrate reduces blood pressure [33; 57; 58; 66–71]. In particular, Kapil et al showed that dietary nitrate supplementation using a daily dose of 250 ml of beet root juice that provided about 400 mg (6.5 mmol) nitrate daily for 4 weeks, lowered pressure in both drug naïve and treated hypertensive subjects [33]. Several studies have also shown that BRJ improves exercise efficiency and/or performance [28–31; 59–61; 64; 71; 72]. Notably, in studies on patients with HFpEF, a larger acute dose of BRJ (12.9 mmol) or smaller dose (6.1 mmol) given daily for a week resulted in improved exercise outcomes [30; 31]. But not all previous studies have been positive. For example, Bondonno et al did not observe an effect of daily dietary nitrate (400 mg) on blood pressure in treated hypertensive individuals [73]. In addition, in our previously published study on patients with HFpEF, we did not observe an effect of a single, acute dose of BRJ on exercise tolerance [31]. Thus one factor that may have contributed to the lack of an effect observed in the studies reported here, as well as the previous, is the size of the nitrate dose. In the current HFpEF study, the dose (6.1 mmol) was the same as in our previous study, but the dosing schedule was lower (three times a week vs 7 times in a week). The dose in the HFpEF study presented here was lower than that in some other studies, for example 6.1 mmol (this HFpEF study) vs 12.9 mmol in the study by Zamani et al [30]. However, the nitrate dose given by Kapil et al was similar to the one in our HFpEF study and less than that in our HTN study.

Another, related factor that may have contributed to lack of additive effects of the BRJ in addition to exercise training is the variability and moderate extent to which plasma nitrite was increased in the participants in our studies (Figure 2). A recent review points out that baseline levels of fitness influence response to dietary nitrate [74] and our patients were sedentary. Moreover, for the both studies, blood was collected only one hour after BRJ consumption and thus plasma nitrite may not have reached a maximum. In previous work we have noted that plasma nitrite is close to a maximum one hour after BRJ consumption [75], but others have seen that the maximum is not reached for two to three hours [57; 67]. Thus, maximum plasma nitrite levels may have been higher than those shown in Figure 2 for the BRJ group. It is also noteworthy that the levels of nitrite measured for the placebo group in the HFpEF study were higher than normally observed (average of 0.47 μM compared to (for example) 0.14 μM for the HTN study). As discussed in the results section, part of the reason for this elevation was due to a single subject with extremely high baseline plasma nitrite. Exclusion of that person brings the average plasma nitrite for the HFpEF placebo group to 0.27 ± 0.17 μM. In addition, the lack of a restricted diet in the HFpEF study could have masked effects of BRJ. Moreover, lack of BRJ effects on plasma nitrite could be due to variability in oral bacteria that reduce nitrate to nitrite. In this regard, in ad hoc analysis of the HTN study we found evidence of a graded relationship between nitrite levels and improvement in exercise time during the exercise test (Figure 5). However, we did not observe a similar relationship in the HFpEF study. It should also be noted that our HTN

study recruited patients with controlled hypertension whereas Kapil et al recruited patients with uncontrolled hypertension. It is possible that medications used by the patients in our HTN study masked any BRJ effects. Of course we recognize that a major factor in our studies is that BRJ was combined with supervised exercise which may also have masked any BRJ effects.

In cases where conversion of nitrate to nitrite is bypassed using either direct nitrite infusion [34] or inhalation [35], administration to patients with HFpEF improved hemodynamics. Success of these studies that bypass oral conversion of nitrate to nitrite may suggest that such methods of administration should be pursued in lieu of BRJ or other dietary nitrate methods. Indeed, levels of plasma nitrite achieved in these studies using inhaled or infused nitrite were an order of magnitude or higher than in our studies using BRJ [34; 35]. However, positive studies involving BRJ in a variety of conditions, including those involving patients with HFpEF in the absence of an exercise intervention [30; 31], underlines the potential of use of dietary nitrate as an inexpensive therapy or co-therapy, at least among those who respond well in terms of producing plasma nitrite.

4.1 Limitations

The studies presented have some limitations. In addition to limitations related to nitrate dose and response (in terms of increases in plasma nitrite) discussed directly above, the major limitation is the number of participants. While 20 and 26 participants may have been sufficient to detect effects of either BRJ or EX alone, the combined effect would probably have to be substantial to detect an added benefit of BRJ on top of EX. In addition a crossover design would likely have added power.

It is also possible that a longer duration of intervention is needed to observe an effect of BRJ. In the HTN study, we did not observe any between group differences in the volume of training completed during each session over the six weeks (data not shown), suggesting that a longer exercise training study would not have led to a different outcome, but we cannot be sure about this based on our data. Perhaps more importantly, more frequent BRJ dosing, particularly in the HFpEF study, may have had additional beneficial effects compared to exercise therapy alone. We have already shown that daily BRJ consumption improves exercise tolerance [31]. The rationale for BRJ dosing only 3X per week was based on the hypothesis that BRJ consumption on the day of exercise training would have an additive or synergistic effect to positive effects of exercise training alone. Whether or not daily dosing with BRJ would have had an additional beneficial effect is still an open question.

Another limitation may be due to the interval of time between when the BRJ was consumed and when testing was administered. BRJ was consumed one hour before exercise training and testing was completed within a couple of hours of consumption. As mentioned above, some studies have observed that plasma nitrite does not peak until 2–3 hours after dietary nitrate consumption. Thus, the effect of BRJ may have been bigger if exercise were started two hours later, but the optimal time is not known (perhaps beginning at one hour post consumption is best as plasma nitrite levels are elevated and would continue to rise during testing). In addition to these limitations, there are some differences in the interventions and outcomes in the two studies described.

5. CONCLUSION

In conclusion, our hypothesis that simultaneous administration of a supervised exercise and dietary nitrate interventions could improve exercise training and subsequent outcomes more than exercise training alone was not supported by the results of the two studies presented here. Further work should be conducted to see if larger nitrate doses or more frequent dosing schedules could result in additive benefits of dietary nitrate intake to those observed through supervised exercise.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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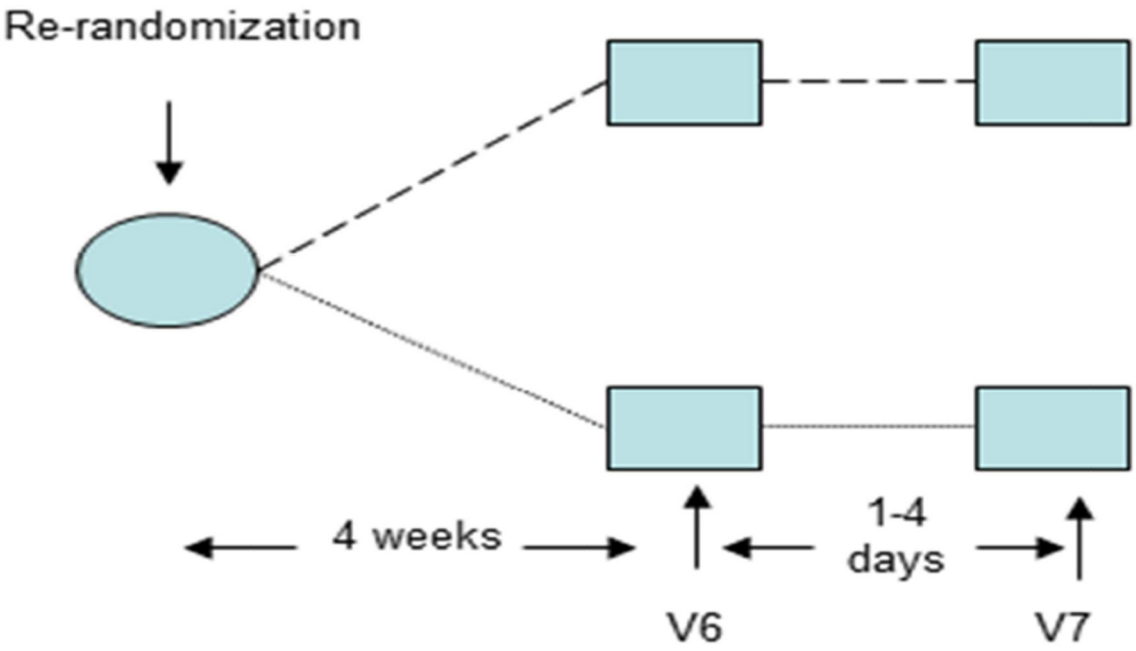
Highlights

- Exercise intervention provided benefits in HTN and HFpEF pilot studies
- Dietary nitrate provided no additional benefit to exercise therapy
- Dose of dietary nitrate used here may have been insufficient



Group 1: 3 day/wk exercise + Placebo

Group 2: 3 day/wk exercise + Beet Juice

V6 = blood pressure, nitrites/nitrates, submaximal constant work rate exercise test

V7 = echo-doppler, maximal exercise test

Baseline values are from the placebo visit during the cross-over design from the previous study [28] or from the screening visit 1 for the Echo-Doppler and maximal exercise test outcomes only

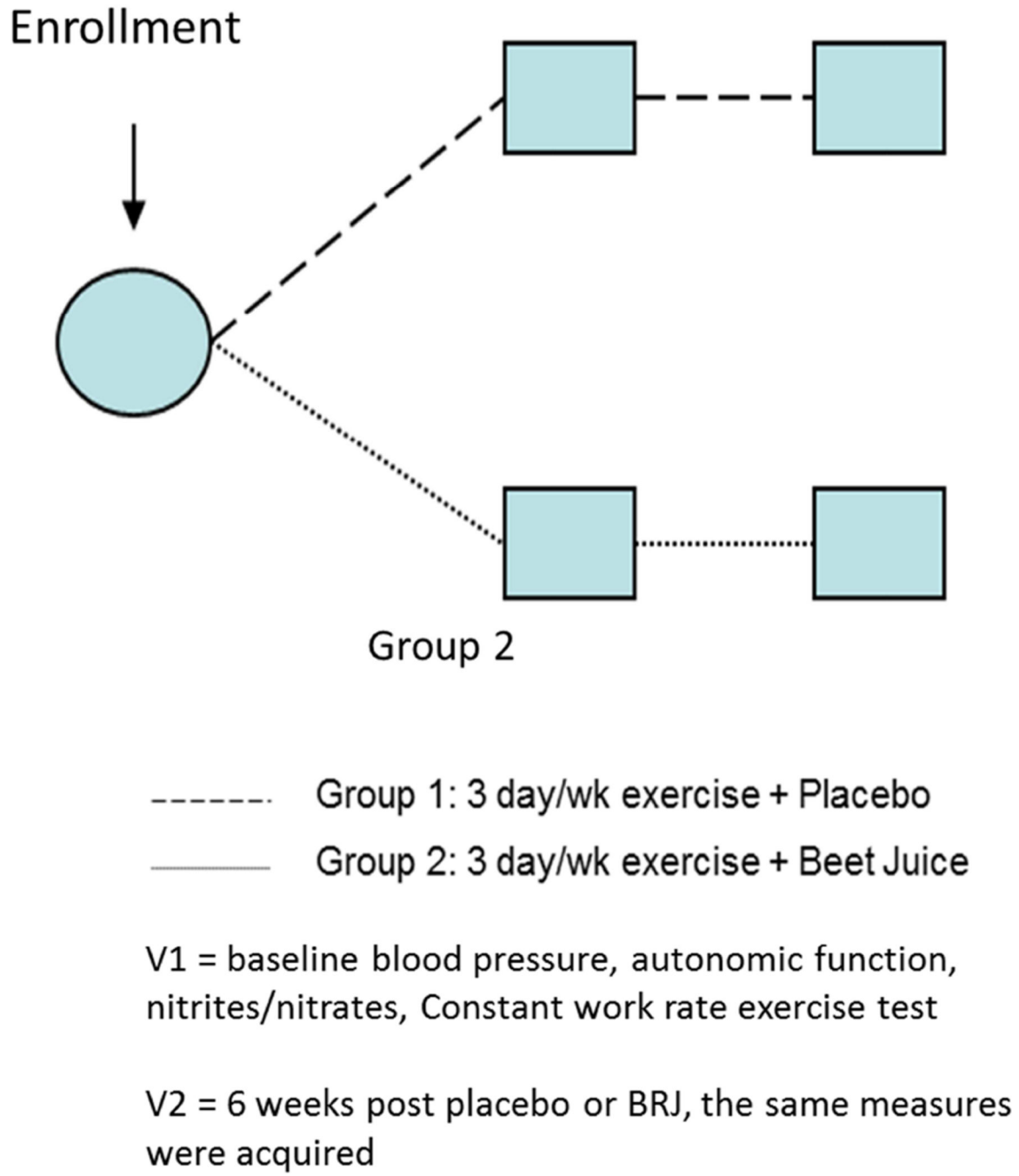


Figure 1.

For HFpEF participants, immediately following the previous study design, V5[31], all subjects were re-randomized to either 4 weeks of EX + Placebo or 4 weeks of EX + BRJ. After the 4-week intervention, subjects completed 2 follow-up visits separated by 1–4 days. Visit 6 consisted of blood pressure measurements, a blood draw, and a submaximal constant workrate exercise test. Visit 7 consisted of a resting Echo-Doppler exam and a maximal exercise test. Baseline values are from the placebo visit during the cross-over design from

the previous study (either V3 or V4) or from the screening visit 1 for the Echo-Doppler and maximal exercise test outcomes only.

For HTN participants, immediately following the initial screening and assessment of exercise performance, cardiovascular and autonomic function at visit 1, all subjects were randomized to either 6 weeks of EX + Placebo or 6 weeks of EX + BRJ. After 6 weeks intervention, subjects returned for follow up visit 2 where the same outcomes were measured again.

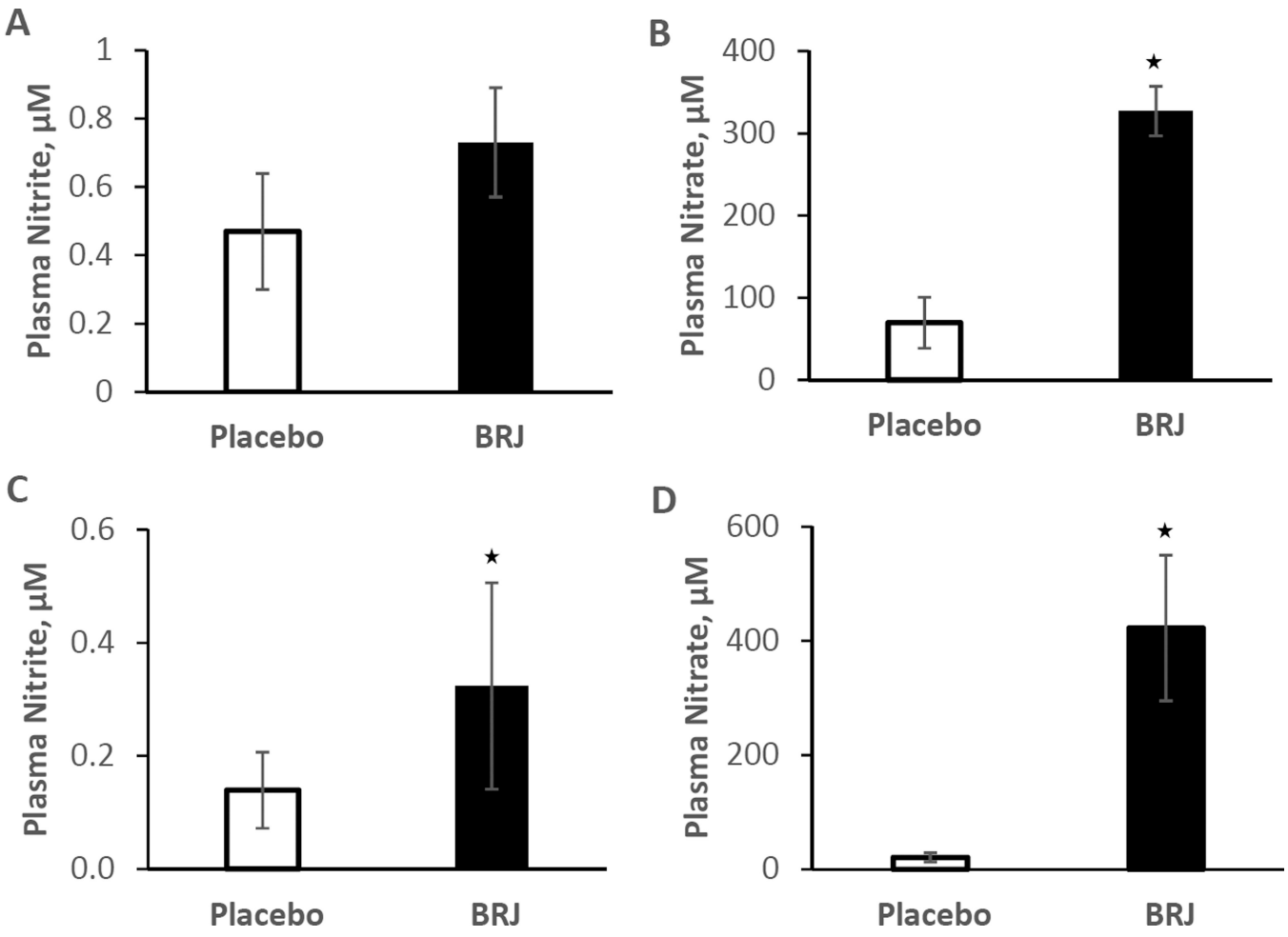


Figure 2.

Plasma NO_x levels at baseline and following beverage consumption. (A) Nitrite from the HFpEF study. (B) Nitrate from the HFpEF study. (C) Nitrite from the HTN study. (D) Nitrate from the HTN study.

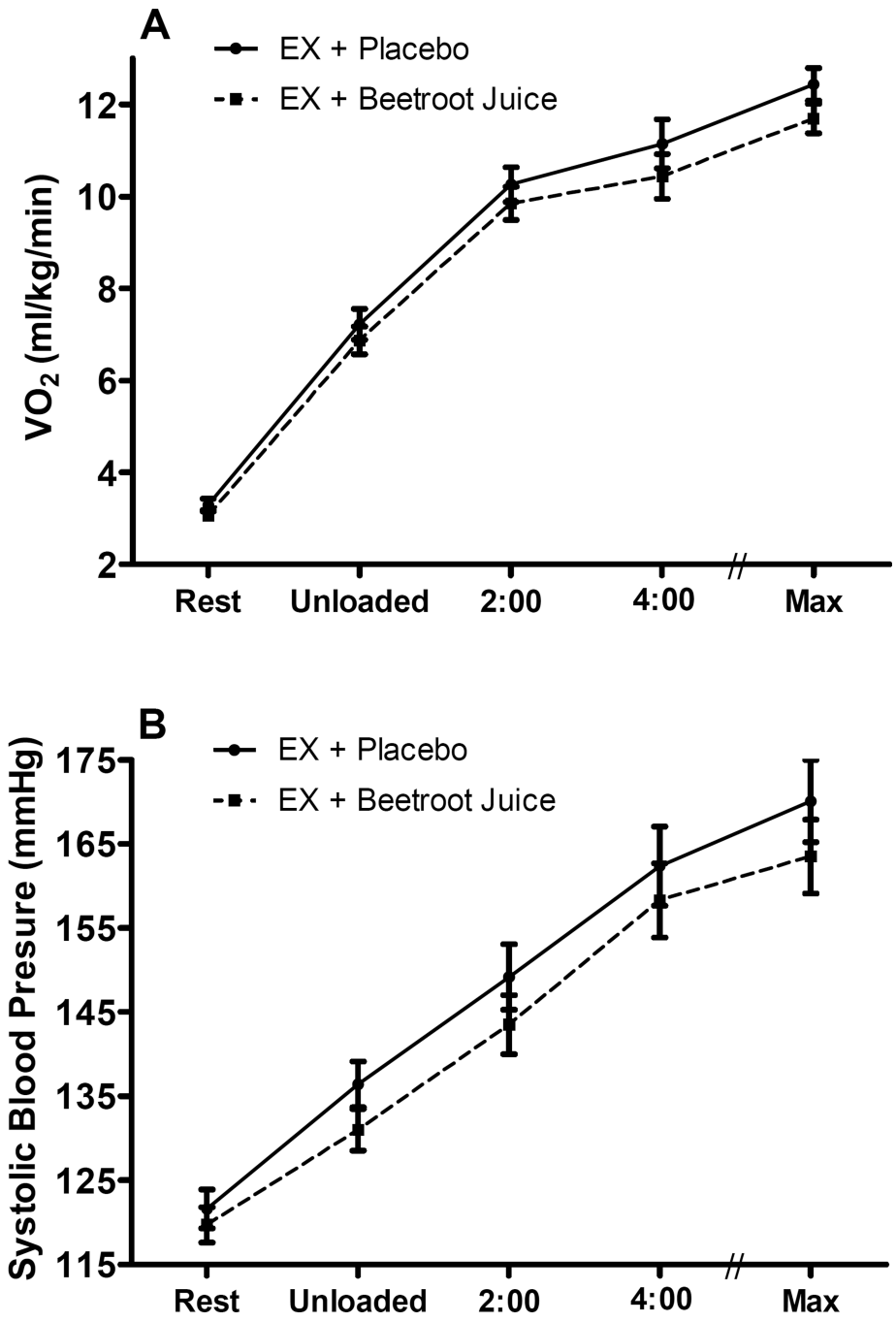


Figure 3.

VO_2 (Panel A) and Systolic Blood Pressure (Panel B) at rest, after unloaded cycling, after 2 and 4 minutes, and at volitional exhaustion (max) from the submaximal constant work rate exercise test in the HFpEF study. Values are the follow-up least square means ± SE (adjusted for the baseline values). The circles and solid line represent the EX + Placebo group and the squares and dotted line represent the EX + Beetroot juice group. There were no differences between Placebo and BRJ groups,

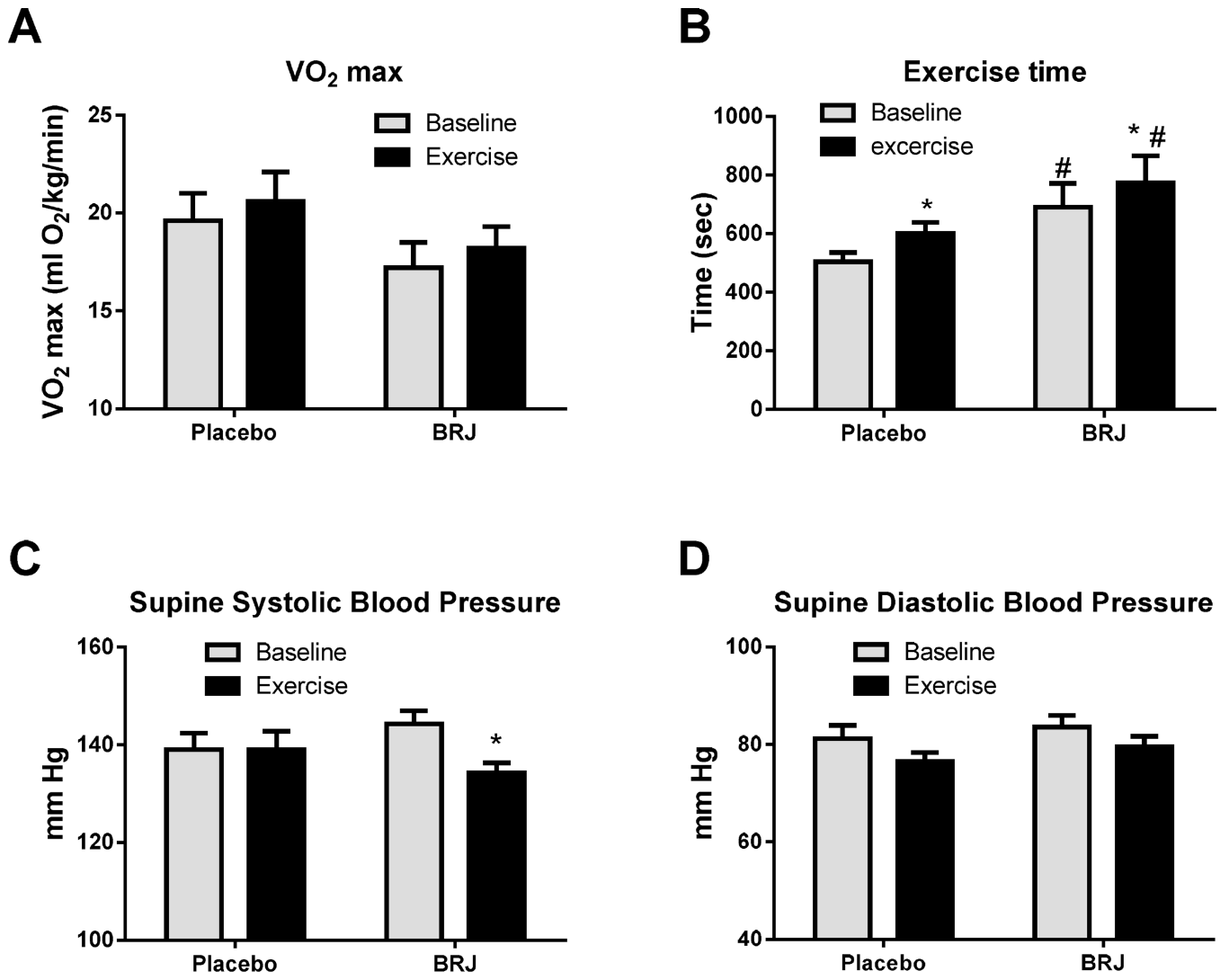


Figure 4.

Effect of exercise (dark color panels) compared to baseline (light color panel) for the HTN study in subjects receiving either high nitrate beet root juice or placebo on VO2 (A); Exercise time (B); Supine systolic blood Pressure (C) Supine diastolic blood pressure (D). Data presented as mean ± SE. * = $p<0.05$ for follow up vs baseline within the same group, # = $p<0.05$ for BRJ vs placebo.

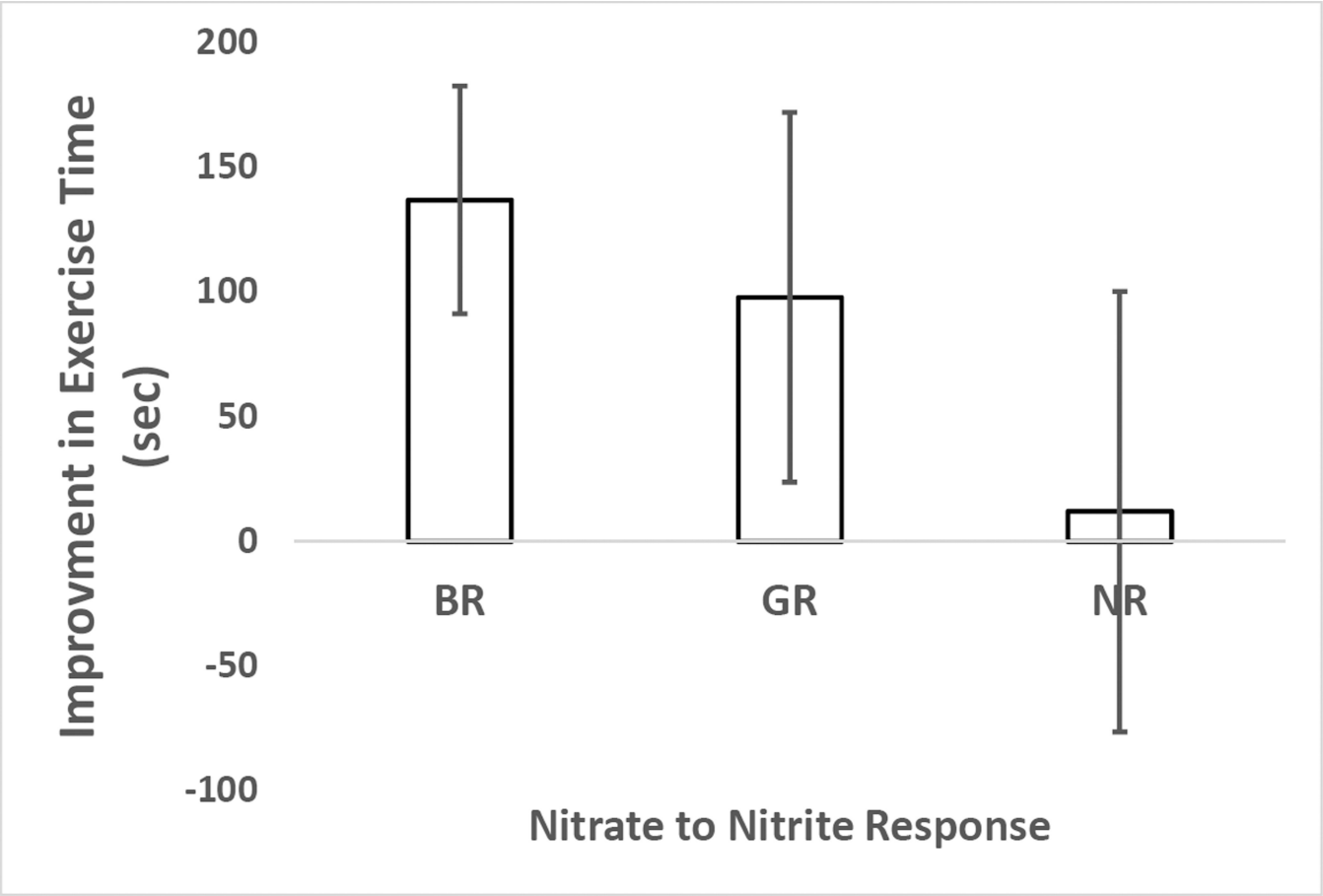


Figure 5.

Relationship between nitrate to nitrite response to improvement in exercise time in the HTN study. Participants were broken into three groups: best responders (BR), good responders (GR), and non-responders (NR) based on change in plasma nitrite following BRJ consumption. Data are the average and standard error of the mean for improvement in exercise time.

Table 1

Participant Characteristics - HFpEF participants

Characteristic	EX + Placebo (N=9)	EX + Beetroot Juice (N=10)	P-Value
Age (years)	70.6 ± 7.6	68.0 ± 6.2	0.43
Female	8 (89%)	8 (80%)	1.0
White	6 (67%)	6 (60%)	1.0
Body Weight (kg)	82.8 ± 15.0	91.9 ± 16.0	0.22
BMI (kg/m^2)	31.5 ± 5.4	33.5 ± 5.8	0.46
NYHA class			
II	8 (89%)	5 (50%)	0.14
III	1 (11%)	5 (50%)	
Diastolic Function			
Normal	2 (22%)	1 (10%)	1.0
Impaired Relaxation	7 (78%)	9 (90%)	
Pseudonormal	0 (0%)	0 (0%)	
Restrictive	0 (0%)	0 (0%)	
e' (cm/s)	5.9 ± 2.0	6.9 ± 1.8	0.26
E/ e' ratio	14.6 ± 7.8	12.0 ± 5.7	0.42
Left atrial diameter (cm)	3.6 ± 0.4	3.8 ± 0.3	0.13
Diabetes mellitus	2 (20%)	5 (50%)	0.35
Hx hypertension	9 (100%)	10 (100%)	1.0
Systolic BP (mmHg)	147 ± 17	144 ± 14	0.74
Diastolic BP (mmHg)	69 ± 12	71 ± 9	0.66
Current medication			
ACE-inhibitors	3 (33%)	3 (30%)	1.0
Diuretics (all)	5 (56%)	7 (70%)	0.65
Loop diuretics	1 (11%)	2 (20%)	1.0
Beta-blockers	2 (22%)	3 (30%)	1.0
Calcium channel blockers	2 (22%)	5 (50%)	0.35
ARB's	2 (22%)	4 (40%)	0.63
Peak VO_2 (ml/kg/min)	12.3 ± 1.7	11.6 ± 2.5	0.50
Peak workload (watts)	58 ± 17	59 ± 26	0.91

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

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

Table 2

Participant Characteristics - HTN participants

Characteristic	EX + Placebo (N=13)	EX + Beetroot Juice (N=13)	P-Value
Age (years)	65.6 ± 7.6	65.0 ± 3.8	0.77
Female	7 (54%)	6 (46%)	0.7
White	10 (77%)	10 (77%)	1.0
Body Weight (kg)	100.5 ± 22.0	95.0 ± 11.0	0.47
BMI (kg/m^2)	35.0 ± 6.5	32.3 ± 4.6	0.26
Systolic BP (mmHg)	129 ± 9	132 ± 10	0.5
Diastolic BP (mmHg)	74 ± 8	76 ± 7	0.6
Current medication			
ACE-inhibitors	5 (38%)	2 (15%)	0.2
Diuretics (all)	4 (30%)	4 (30%)	1.0
Beta-blockers	2 (15%)	2 (15%)	1.0
Calcium channel blockers	0 (0%)	0 (0%)	1.0
ARB's	2 (15%)	2 (15%)	1.0
Peak VO_2 (ml/kg/min)	19.6 ± 5.0	17.2 ± 4.6	0.25

Data presented as mean ± SD or number (%)

Abbreviations- BMI: body mass index; BP: blood pressure; ACE: angiotensin converting enzyme; ARB: angiotensin receptor blocker

Table 3

Submaximal Constant Workload Exercise Test – HFpEF participants

Variable	EX + Placebo			EX + Beetroot Juice			P-value
	Baseline	Follow-up	LS Mean ± SE	Baseline	Follow-up	LS Mean ± SE	
Submax Exercise Test (n)	8	8	8	10	10	10	
Exercise time at constant work rate, sec	369 ± 149	520 ± 257*	531 ± 68	384 ± 129	483 ± 258	475 ± 60	0.55
Resting systolic blood pressure, mmHg	136 ± 16	122 ± 3*	122 ± 2	132 ± 12	119 ± 9*	120 ± 2	0.56
Resting diastolic blood pressure, mmHg	77 ± 11	73 ± 11	72 ± 3	72 ± 9	70 ± 8	72 ± 2	0.94
At exhaustion:							
VO_2, ml/kg/min	12.2 ± 1.5	12.7 ± 2.1	12.4 ± 0.4	11.6 ± 2.6	11.5 ± 2.4	11.7 ± 0.3	0.14
VO_2, ml/min	1017 ± 275	1050 ± 281	1085 ± 28	1083 ± 355	1065 ± 344	1037 ± 25	0.22
VCO_2, ml/min	1233 ± 406	1182 ± 391	1187 ± 48	1243 ± 448	1215 ± 453	1211 ± 43	0.72
Respiratory exchange ratio	1.20 ± 0.10	1.12 ± 0.08*	1.10 ± 0.03	1.14 ± 0.07	1.12 ± 0.10	1.14 ± 0.03	0.36
Heart rate, bpm	123 ± 16	119 ± 16	116 ± 3	118 ± 24	116 ± 25	118 ± 3	0.62
Systolic blood pressure, mmHg	172 ± 12	173 ± 13	170 ± 5	163 ± 19	161 ± 19	163 ± 4	0.34
Diastolic blood pressure, mmHg	79 ± 12	81 ± 9	80 ± 2	76 ± 8	75 ± 7	76 ± 2	0.15

Data presented as mean ± SD or number (%) and the least-square (LS) mean ± SE of the follow-up value adjusted for the baseline value. P-value corresponds to comparison of the least-square mean (LS Mean). Mean constant work rate = 46 W for EX + Placebo and 44 W for EX + Beetroot juice. Exercise time excludes the 2-minute unloaded pedaling period.

*p<0.05 for within group change by paired samples t-test (follow-up –baseline).

Table 4

Maximal Exercise Test – HFpEF participants

Variable	EX + Placebo			EX + Beetroot Juice			P-value
	Baseline	Follow-up	LS Mean ± SE	Baseline	Follow-up	LS Mean ± SE	
Peak Exercise (n=)	9	9	9	10	10	10	
Exercise time (sec)	462 ± 97	502 ± 99 *	507 ± 19	473 ± 153	500 ± 162	495 ± 18	0.62
Workload (W)	58 ± 17	64 ± 17	65 ± 3	59 ± 26	63 ± 27	62 ± 3	0.52
VO_2 (ml/kg/min)	12.3 ± 1.7	12.2 ± 1.9	11.9 ± 0.4	11.6 ± 2.5	11.6 ± 3.0	12.0 ± 0.4	0.89
VO_2 (ml/min)	1014 ± 246	1012 ± 244	1048 ± 38	1082 ± 347	1082 ± 390	1049 ± 36	0.99
VCO_2 (ml/min)	1203 ± 321	1246 ± 352	1276 ± 66	1263 ± 480	1284 ± 504	1256 ± 63	0.83
RER	1.18 ± 0.13	1.23 ± 0.06	1.22 ± 0.02	1.15 ± 0.10	1.18 ± 0.07	1.18 ± 0.02	0.18
HR (bpm)	117 ± 19	120 ± 18	122 ± 5	123 ± 25	123 ± 22	121 ± 4	0.80
SBP (mmHg)	174 ± 7	170 ± 11	169 ± 4	165 ± 17	165 ± 14	167 ± 4	0.76
DBP (mmHg)	77 ± 6	76 ± 9	76 ± 3	77 ± 6	75 ± 10	75 ± 3	0.82
VE/ VCO_2 slope	31.4 ± 5.2	33.2 ± 6.0	32.4 ± 1.1	30.0 ± 4.3	31.7 ± 5.6	32.4 ± 1.1	0.97
VAT (ml/min)	663 ± 195	669 ± 133	686 ± 35	702 ± 243	734 ± 270	718 ± 33	0.52

Data presented as mean ± SD or number (%) and the least-square mean (LS Mean) ± SE of the follow-up value adjusted for the baseline value. P-value corresponds to comparison of the least-square mean.

Abbreviations- RER: respiratory exchange ratio; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; VE: minute ventilation; VAT: ventilatory anaerobic threshold.

*p<0.05 for within group change by paired samples t-test (follow-up – baseline).

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Table 5

Physical performance and Hemodynamic Measures – HTN participants

Variable	EX + Placebo			EX + Beetroot Juice			P-Value
	Baseline	Follow-up	LS Mean ± SE	Baseline	Follow-up	LS Mean ± SE	
Graded Exercise Test (n)	**13**	**13**	**13**	**14**	**14**	**14**	
VO_{2peak}	17.0 ± 4.8	18.2 ± 4.1	19.4 ± 0.5	19.7 ± 5.1	20.6 ± 5.4	19.4 ± 0.5	0.98
Exercise time (sec)	504 ± 109	*601 ± 133	699 ± 37	691 ± 300	*773 ± 343	682 ± 35	0.74
Supine resting SBP	139 ± 12	139 ± 13	140 ± 4	144 ± 10	*134 ± 14	133 ± 4	0.22
Supine resting DBP	81 ± 10	77 ± 6	77 ± 2	84 ± 8	79 ± 8	79 ± 1	0.44
Peak SBP	186 ± 22	186 ± 15	189 ± 5	192 ± 22	192 ± 28	190 ± 5	0.82
Peak DBP	88 ± 10	82 ± 9	81 ± 3	86 ± 13	83 ± 17	84 ± 3	0.51
24 hr ABPM (n)	**12**	**12**		**12**	**12**		
24 hour SBP	128 ± 8	127 ± 7	129 ± 2	133 ± 11	131 ± 10	130 ± 2	0.88
24 hour DBP	74 ± 9	72 ± 8	73 ± 1	76 ± 7	75 ± 7	74 ± 1	0.33
24 hour MBP	93 ± 7	92 ± 6	93 ± 1	95 ± 7	95 ± 7	74 ± 1	0.65
24 hour HR	73 ± 9	72 ± 8	72 ± 1	72 ± 11	69 ± 7	70 ± 1	0.28
Day time SBP	131 ± 8	130 ± 6	131 ± 2	134 ± 10	135 ± 9	134 ± 2	0.24
Day time DBP	76 ± 10	76 ± 8	76 ± 1	77 ± 7	78 ± 7	78 ± 1	0.35
Day time MBP	96 ± 9	95 ± 6	95 ± 1	97 ± 7	98 ± 7	98 ± 1	0.18
Day time HR	74 ± 9	75 ± 11	74 ± 2	74 ± 12	70 ± 7	70 ± 2	0.21
Night time SBP	125 ± 8	126 ± 10	128 ± 2	131 ± 13	127 ± 12	125 ± 2	0.36
Night time DBP	71 ± 8	70 ± 9	71 ± 2	74 ± 9	72 ± 7	71 ± 2	0.99
Night time MBP	90 ± 7	90 ± 9	91 ± 2	94 ± 9	91 ± 8	90 ± 2	0.62
Night time HR	72 ± 9	72 ± 9	71 ± 2	71 ± 11	68 ± 8	69 ± 2	0.32
Hemodynamic measures (n)	**12**	**12**		**13**	**13**		
SV (ml/beat)	92 ± 26	101 ± 23	105 ± 5	105 ± 17	107 ± 15	104 ± 4	0.82
CO (L/min)	5.9 ± 1.6	6.0 ± 1.4	6.3 ± 0.2	6.6 ± 0.9	6.5 ± 0.8	6.2 ± 0.2	0.96
SVR (dyne sec cm^{-5})	1321 ± 399	1215 ± 331	1148 ± 48	1143 ± 196	1117 ± 209	1179 ± 46	0.65

Variable	EX + Placebo			EX + Beetroot Juice			P-Value
	Baseline	Follow-up	LS Mean ± SE	Baseline	Follow-up	LS Mean ± SE	
TAC (ml/mm Hg)	15.3 ± 3.9	*18.4 ± 5.3	19.4 ± 1.1	18.3 ± 5.3	*20.5 ± 4.1	19.6 ± 1.0	0.91
VI (/1000/s)	35.5 ± 11.0	38.3 ± 10.4	39.5 ± 2.3	39.8 ± 9.4	37.5 ± 8.6	36.3 ± 2.2	0.33
AI (/100/s^2)	67.1 ± 18.8	63.9 ± 14.6	63.3 ± 3.8	64.5 ± 21.4	61.2 ± 17.4	61.8 ± 3.6	0.79
LCWI (kg-m /m^2/min)	3.48 ± 0.89	3.35 ± 0.71	3.49 ± 0.16	4.09 ± 0.57	3.78 ± 0.49	3.65 ± 0.15	0.48

Data presented as mean ± SD or number (%) and the least-square mean (LS Mean) ± SE of the follow-up value adjusted for the baseline value. P-value corresponds to comparison of the least-square mean.

*p<0.05 for follow up vs baseline within the same group.

Abbreviations- ABPM: ambulatory blood pressure measurements; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; HR: heart rate; SV: stroke volume; CO: cardiac output; SVR: systemic vascular resistance; TAC: total arterial compliance; VI; velocity index; AI; acceleration index; LCWI; left cardiac work index; Kg-m/m^2: kilogram-meter per meter squared.

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Table 6

Table 6 - Resting Echo-Doppler– HFpEF participants

Variable	EX + Placebo			EX + Beetroot Juice			P-value
	Baseline	Follow-up	LS Mean ± SE	Baseline	Follow-up	LS Mean ± SE	
Echo-Doppler (n=)	9	9	9	8	8	8	
LV Mass (g)	155.3 ± 21.8	159.2 ± 30.4	170.8 ± 5.1	192.0 ± 45.3	181.6 ± 36.8	168.5 ± 5.5	0.77
E peak (cm/s)	75 ± 18	79 ± 19	80 ± 6	78 ± 28	86 ± 24	85 ± 6	0.52
A peak (cm/s)	98 ± 26	99 ± 21	97 ± 4	94 ± 26	101 ± 26	103 ± 4	0.27
E/A ratio	0.78 ± 0.18	0.81 ± 0.17	0.84 ± 0.04	0.92 ± 0.50	0.89 ± 0.30	0.85 ± 0.04	0.99
e' (cm/s)	5.9 ± 2.0	5.9 ± 1.6	6.2 ± 0.3	6.7 ± 2.0	6.3 ± 2.0	6.0 ± 0.3	0.66
E/ e' ratio	11.9 ± 4.9	14.4 ± 5.3	13.7 ± 1.4	11.6 ± 5.0	15.2 ± 8.0	16.0 ± 1.5	0.63*
E deceleration time (ms)	310 ± 108	264 ± 50	259 ± 15	245 ± 45	226 ± 34	231 ± 16	0.23
LA diameter (cm)	3.6 ± 0.4	3.6 ± 0.4	3.7 ± 0.03	3.8 ± 0.4	3.8 ± 0.3	3.7 ± 0.03	0.56

Data presented as mean ± SD or number (%) and the least-square (LS) mean ± SE of the follow-up value adjusted for the baseline value. P-value corresponds to comparison of the least-square mean.

* p-value is following logarithmic transformation of this highly skewed variable.

Abbreviations- LV: left ventricular; E: early mitral velocity; A: atrial mitral velocity; e': mitral annulus velocity (septal); LA: left atrial