

ACUTE BEETROOT SUPPLEMENTATION MAY IMPROVE BLOOD PRESSURE BUT NOT EXERCISE ECONOMY IN FEMALE MASTERS SWIMMERS

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Abstract

It is known that beetroot supplements may improve exercise economy and blood pressure, but this has mainly been studied in males. Given that older female athletes are underrepresented in the literature, we aimed to determine if acute beetroot supplementation (BRS) improves exercise economy and blood pressure in masters female athletes (swimmers) during a treadmill exercise test. Methods: 11 participants (57.8±10.5 y) underwent 2 randomized, double-blinded trials, ingesting beetroot (BE) or placebo (PL). Salivary NO₂⁻ (sNO₂⁻) and blood pressure (BP) were measured pre-ingestion (Base), pre-exercise (Pre), and 5 min post-exercise (Post). Oxygen consumption (VO₂) was measured during the modified Balke test until HR reached 85% of age-predicted maximum. Exercise economy (ExEc) was defined as the average relative VO₂ during min 3-4.5 of the test. sNO₂⁻ was determined using NO₂⁻ detection strips. Results: sNO₂⁻ increased from Base to Post in BE vs PL (32.5±7.0 vs 2.7±3.9% change, *p* = 0.001). No treatment differences existed for ExEc (BE: 15.51±0.47 vs PL: 15.71±0.53 ml·kg⁻¹·min⁻¹, *p* = 0.48). Diastolic BP was significantly lower in BE vs PL (Base: 74.6±1.7 vs 73.2±2.3, Pre: 73.6±1.8 vs 74.5±2.1, Post: 74.5±1.7 vs 76.1±2.2 mmHg, *p* = 0.03, treatment by time). Conclusion: Acute BRS lowered diastolic BP, but did not improve exercise economy in these trained, normotensive athletes. Further research is warranted in menopausal and post-menopausal females, including those who are hypertensive, and in other female masters athlete groups.

Keywords: older athletes, dietary nitrate, treadmill exercise

1 Introduction

Beetroot supplements are popular among athletes who seek to improve endurance exercise performance and older adults who desire to lower blood pressure. Beetroot supplements (BRS) are high in dietary nitrate (NO₃⁻), which circulates in the plasma after ingestion; a portion of this NO₃⁻ enters the entero-salivary circulation and is concentrated in the saliva (Spiegelhalter et al., 1976). The salivary NO₃⁻ is then reduced to nitrite (NO₂⁻) by bacteria on the surface of the tongue (Webb et al., 2008). When this NO₂⁻ is swallowed and enters the stomach, some is further reduced in the acidic environment to nitric oxide (NO), while some enter the systemic circulation, increasing circulating NO (Lundberg Govoni, 2004). NO is a powerful signaling molecule that lowers resting blood pressure by increasing vasodilation and may reduce the oxygen cost of submaximal exercise, improving exercise perfor-

mance (Jones, 2014).

The effects of BRS on exercise economy have been widely investigated, with several investigations reporting improvements (i.e., reduced oxygen cost of exercise) with BRS (Bailey et al., 2009; Larsen et al., 2007; Pinna et al., 2014; Waldron et al., 2018), while others have not found significant improvements with BRS compared to placebo (Rokkedal-Lausch et al., 2021; Wickham et al., 2019). The blood pressure-lowering effects of BRS have also been widely studied, with many investigations reporting that BRS is effective in lowering resting blood pressure (Bailey et al., 2009; Lansley et al., 2011b; Stanaway et al., 2019; Vanhatalo et al., 2010; Waldron et al., 2018; Webb et al., 2008).

Despite the plethora of research on the effects of BRS on exercise economy and on blood pressure, there are notable gaps. Firstly, most investigations have been conducted using mostly

or exclusively male participants. Females, especially older female athletes, are vastly underrepresented in the currently published investigations concerning dietary NO₃⁻ supplementation (Wickham Spriet, 2019). Therefore, studying BRS in females could lead to a better understanding of the effects of BRS in this population.

In addition, many of the studies of BRS are conducted using recreationally active participants rather than well-trained individuals (Bailey et al., 2009; Perez et al., 2019; Vanhatalo et al., 2010; Waldron et al., 2018; Wickham et al., 2019). Of the studies that focus on trained athletes, most used cyclists (Lansley et al., 2011a; Rokkedal-Lausch et al., 2021) or runners (Boorsma et al., 2014; de Castro et al., 2019). A few studies have examined BRS with swimmers (Esen et al., 2019; Pinna et al., 2014; Pospieszna et al., 2016), but the participants were generally young (average age of 20-22 yrs.) (Esen et al., 2019; Lowings et al., 2017; Pospieszna et al., 2016), or were all male (Pinna et al., 2014). To our knowledge, the effects of BRS on exercise economy and blood pressure have not been studied exclusively in female masters (defined as age 35 yrs and older) athletes, especially in those that are swimmers.

Therefore, the primary purpose of this study was to determine if acute BRS improved the oxygen cost of submaximal exercise (i.e., exercise economy, ExEc) in female masters swimmers during an incremental treadmill test. We also aimed to determine if acute BRS lowered blood pressure (BP). We hypothesized that acute BRS would improve exercise ExEc and reduce pre- and post-exercise BP compared to a placebo treatment in our population of trained female masters swimmers.

2 Methods

2.1 Experimental Design

This study used a randomized, double-blind, crossover design. Participants underwent two randomly ordered, double-blinded trials in which they ingested either 10 oz of BRS (BE) or placebo (PL) 30 min before performing a modified Balke treadmill test. Baseline BP and salivary NO₂⁻ levels were measured pre-ingestion (Base), 25 min after ingestion, which was 5 min pre-exercise (Pre), and post-exercise (Post). Oxygen consumption (VO₂) was measured every 15 sec, and HR and RPE were measured every 60 sec of the treadmill test until participants reached 85% of their APMHR. Dependent variables were salivary NO₂⁻ response, BP, HR, ExEc, RPE, and treadmill test time.

The protocol for the experimental trials is shown in Figure 1.

2.2 Participants

Eleven female masters swimmers (age 57.8±10.4 yrs.; height: 167.8±5.5 cm; body mass: 66.7±11.0 kg) enrolled in and completed the study. Each had trained consistently and competed in swimming events for at least the last year. Participants were recruited via emails to local masters swimming groups and clubs in the Minneapolis/Saint Paul, Minnesota, USA, metropolitan area. All participants provided voluntary written informed consent to participate in the study, which was approved by the Hamline University Institutional Review Board (2019-05-27ET). Prior to testing, participants were screened for any cardiorespiratory or musculoskeletal issues that would put them at greater risk of an adverse event through a health history questionnaire and a resting electrocardiogram (ECG), which was read by a cardiologist. Prior to recruitment, a power analysis was performed using G-Power 3.1.9.2 software (Dusseldorf University, Germany; (Faul et al., 2007)). The minimum number of participants needed for a two-tailed alpha level of 0.05 and desired power value of 0.80 was 10.

2.2.1 Pre-Trial Diet and Nitrate/Nitrite Washout

Participants completed a 3-day food and exercise log before the first trial and were asked to replicate the same diet and exercise schedule during the 24-hr period prior before the second trial. They were asked to avoid nitrate and nitrite-containing foods for the 24 hrs. prior to each trial, and a list of nitrate and nitrite-containing foods was provided to them at their screening session. Participants were also asked to avoid mouthwash and chewing gum for 24 hrs. prior to each trial and compliance was checked by investigators.

2.2.2 Experimental Beverages

The BE (BeetElite, Human^N Co, Austin, TX, USA) and PL ingredients were obtained in powder form and mixed in the laboratory by a laboratory member not involved in data collection. Both were mixed into 10 oz of water in an opaque sports bottle. The placebo beverage matched the BE in energy and electrolyte content and in color.

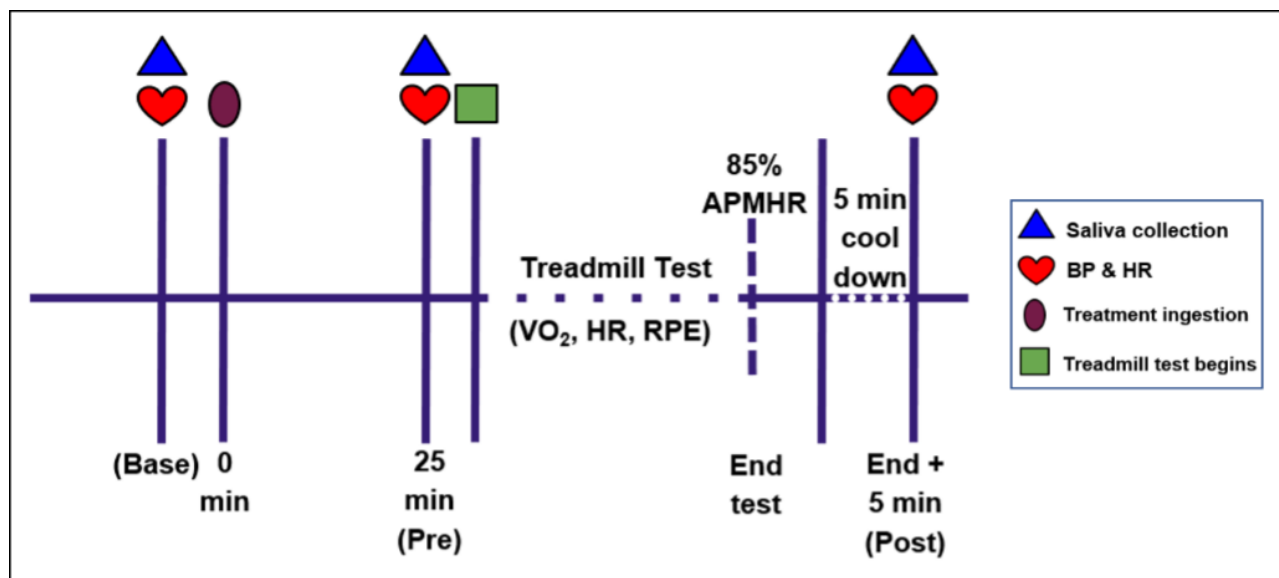


Figure 1: Experimental protocol

Notes. Participants ingested either 10 oz of beetroot supplement or placebo 30 min before performing a modified Balke treadmill test. The 3-time points (Base, Pre, and Post) for saliva sampling and seated blood pressure and heart rate measurements are shown. Peak VO_2 , HR, and RPE were recorded at the end of the treadmill test.

2.3 Measurements

2.3.1 Salivary NO_2^- Response

The salivary NO_2^- response was used as an indirect indicator of the systemic NO response to the treatments. Saliva samples (0.5 mL) were taken via passive drool into a collection tube (Salimetrics, State College, PA) at each of the 3-time points (Base, Pre, Post). Changes from baseline in salivary NO_2^- response was determined using commercially available NO indicator strips (Nitric Oxide Indicator Strips, Human^N, Austin, TX) and a quantification and analysis protocol developed in our lab. Because the manufacturer's instructions were not sufficiently standardized for laboratory use, we previously developed a process to ensure validity and reliability of test strips across the 3-time points.

Briefly, this protocol was as follows: 2 min prior to each saliva collection, participants rinsed their mouths with a small amount of water, and the collection was taken via passive drool. A test strip was exposed to the sample for 2 sec. Images were taken at 60-sec post-collection with an iPad Mini on an image capture station (Figure 2A). Each sample test strip pad was placed between a standard (dark pink intensity) and a background reference (white pad) strip, and an image showing all 3 test strip pads was taken at each time point (Figure 2B). After each trial, the images were downloaded to a lab computer,

and the color intensity of the standard, sample, and background pads in each image was analyzed using ImageJ (Schneider et al., 2012). The ImageJ results were exported into Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA), where the background values were subtracted, and the absolute sample color intensity values were converted to percentage of the standard intensity value.

2.3.2 Blood Pressure

Blood pressure (BP) was measured manually at the 3-time points (Base, Pre, Post) using a cuff and sphygmomanometer (752M Mobile Aneroid; American Diagnostic Corporation, Hauppauge, NY, USA). Participants rested, seated, for 5 mins before BP was measured in the dominant arm. 3 mins later, BP measurement was repeated on the other arm. The highest of the 2 values was used for analysis.

2.3.3 Treadmill Testing

The modified Balke protocol was performed on a Trackmaster TMX425C treadmill (Full Vision, Newton, KS, USA) and consisted of a 2 min warm-up at 3.0 mph, then a testing portion at 3.5 mph in which the grade increased by 2% every 2 min until the participant reached 85% of their age-predicted maximum heart rate (APMHR) using the formula ($HR_{max} = 208 - 0.7 \times \text{age}$) (Tanaka

et al., 2001); the duration of this testing portion was recorded as the treadmill test time. Once APMHR was achieved, the incline was lowered to 0% grade and speed was reduced to 2.0 mph for a 5-minute cool down. During each trial, the laboratory temperature was maintained at approximately 21°C, and a fan was directed toward the participant to reduce thermal stress. Consistent verbal encouragement was given to all participants by the same investigators during each trial.

2.3.4 Exercise Economy

Oxygen consumption (VO_2) was continuously measured throughout the treadmill test, with values recorded every 15 sec. Participants breathed through a Hans Rudolph valve, and expired gases were directed to a mixing chamber for the analysis of oxygen and carbon dioxide (ParvoMedics TrueOne 2400, Parvo Medics, Sandy, UT, USA). Exercise economy (ExEc) was defined as the average VO_2 relative to body mass ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) between minutes 3 and 4.5 of the treadmill test (Losnegard et al., 2014).

2.3.5 Heart Rate

HR was recorded using a heart rate monitor (Polar Electro Oy, Kempele, Finland). HR was measured at each of the 3 time points (Base, Pre, Post), as well as every minute during the treadmill test and the cool down.

2.3.6 Rating of Perceived Exertion

RPE was assessed every 2 min during the treadmill test using a 1-10 scale, with 1 indicating no effort at all, and 10 indicating maximal effort. A color-coded chart was displayed on the treadmill control panel and its use was explained to the participants before each trial. This allowed participants to relay their RPE physically by pointing.

2.4 Statistical Analyses

ExEc, RPE, and treadmill time were analyzed using two-tailed paired t-tests. Salivary NO_2 -levels, SBP, DBP, and HR were analyzed with two-way (treatment x time) repeated measures ANOVA. Post hoc analysis was performed using a Bonferroni correction when significance was found. The significance level for all analyses was determined at $p \leq 0.05$. All data were expressed as mean \pm SE. SPSS Version 26 software (IBM Corp., Armonk, NY) was used for all statistical analysis.

3 Results

3.1 Blood pressure

As shown in Figure 3A, no treatment differences in systolic BP were found between BE and PL (Base: 116.6 ± 1.5 vs. 115.5 ± 1.6 , Pre: 115.0 ± 1.7 vs. 116.0 ± 1.7 , Post: 116.5 ± 1.4 vs. 118.3 ± 1.5 mmHg, respectively, $p = 0.71$), although trends towards significance existed for differences by time ($p = 0.059$), and treatment by time ($p = 0.053$). Significant differences in diastolic BP Figure 3B) existed between BE and PL by time (Base: 74.6 ± 1.7 vs. 73.2 ± 2.3 , Pre: 73.6 ± 1.8 vs. 74.5 ± 2.1 , Post: 74.5 ± 1.7 vs. 76.1 ± 2.2 mmHg, $p = 0.035$) and treatment by time ($p = 0.026$), although treatment-only differences were not significant ($p = 0.79$).

3.2 Salivary NO_2 -

As shown in Figure 4, salivary NO_2 - levels, an indirect indicator of the systemic NO response, increased significantly from Base in BE (Base: $26.1\% \pm 3.1\%$, Pre: $52.0\% \pm 4.2\%$, Post $58.5\% \pm 6.8\%$), but not in PL (Base: $25.4\% \pm 2.1\%$, Pre: $31.9\% \pm 3.7\%$, Post: $28.1\% \pm 3.7\%$, $p = 0.001$), with significant time and treatment by time effects ($p = 0.000$).

3.3 Exercise Economy

There were no significant differences between treatments in ExEc (BE: 15.51 ± 0.47 vs. PL: 15.71 ± 0.53 $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $p = 0.48$, Fig. 5A). There was also no significant difference in treadmill test time between BE and PL (BE: 15.5 ± 1.9 vs. PL: 15.4 ± 1.8 min, $p = 0.92$, Fig. 5B).

3.4 Heart Rate

The heart rate response to acute BRS did not differ between BE and PL (Base: 62.0 ± 2.4 vs 63.6 ± 2.1 , Pre: 63.2 ± 2.5 vs 65.4 ± 2.2 , and Post: 72.6 ± 3.2 vs 74.6 ± 2.4 bpm, respectively, $p = 0.12$), although the time effect was significant as expected ($p = 0.00$). The treatment by time interaction was not significant ($p = 0.86$).

3.5 Rating of Perceived Exertion

Peak RPE was not significantly different between treatments (BE: 6.2 ± 0.5 vs. PL: 6.5 ± 0.5 , $p = 0.25$).

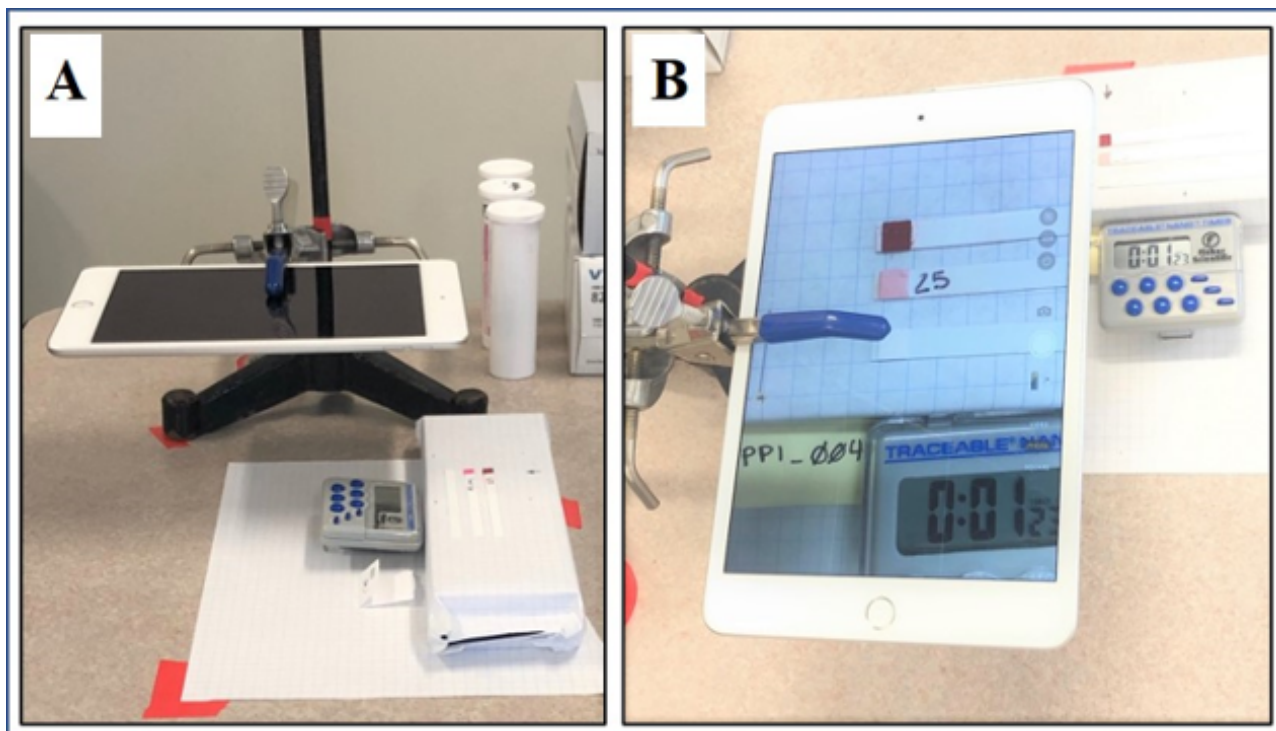


Figure 2: Image capture system for determination of the salivary nitrite response

Notes. A. Side view of the image capture station. B. Example of an image on the iPad Mini screen, as is captured during an experimental trial. The standard is the top (darkest) pad, the reference pad is the bottom, and the test strip (labeled) is in the middle. Quantification and analysis were performed post-trial using ImageJ.

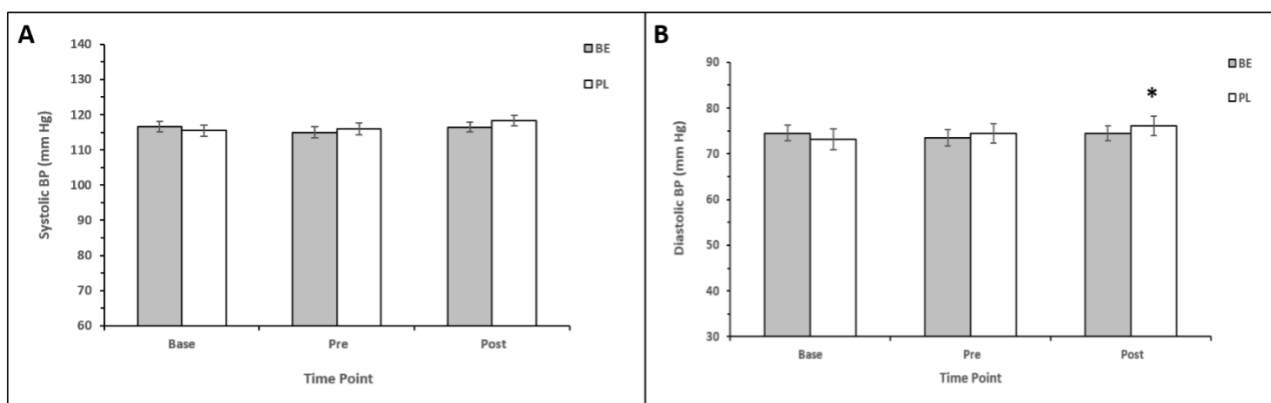


Figure 3: Systolic (A) and diastolic (B) blood pressure was measured at 3 time points during each trial (Base, Pre, and Post).

Notes. A. Systolic blood pressure. B. Diastolic blood pressure (*treatment by time, $p = 0.026$); A significant time effect was present for diastolic blood pressure ($p = 0.035$), whereas there was a trend toward a significant time effect for systolic blood pressure ($p = 0.059$). The systolic blood pressure treatment by time interaction also trended toward significance at $p = 0.053$. Values are mean \pm SE.

4 Discussion

The primary purpose of this study was to determine if acute beetroot supplementation (BRS) lowered the oxygen cost of exercise (improved ExEc) in female masters swimmers during an in-

cremental submaximal treadmill test. We also aimed to determine if acute BRS lowered BP in our study group. The most noteworthy finding of this study was that although ExEc did not differ between the BRS and placebo treatments, post-exercise diastolic BP was significantly lower with

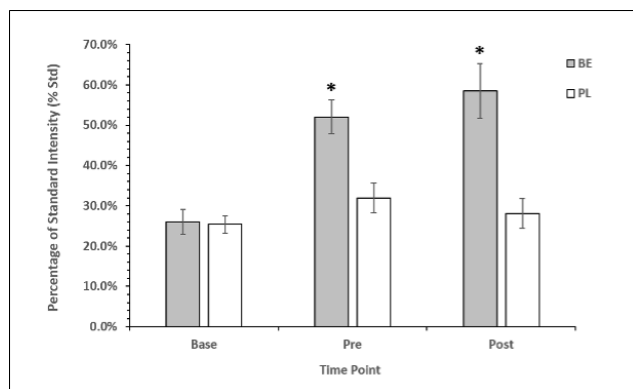


Figure 4: Salivary nitrite response at 3-time points during each trial (Base, Pre, and Post).

Note. Significant effects were found for treatment (* $p = 0.012$), time ($p = 0.000$), and treatment by time ($p = 0.001$). Values are mean \pm SE.

the BRS treatment (BE) compared to placebo (PL). To our knowledge, this was the first investigation to examine the effects of BRS on ExEc and BP in female masters athletes (i.e., over the age of 35 yrs.).

In the present study, BRS did not improve ExEc, which is in disagreement with several other studies that reported either improved ExEc (Muggeridge et al., 2013; Vanhatalo et al., 2010) or improved power output at the same VO_2 (Lansley et al., 2011a) with acute BRS compared to placebo. However, these 3 previous investigations used either exclusively or mostly male participants. Our finding of no improvement in ExEc with BRS agrees with that of Wickham et al. (Wickham et al., 2019), who also used a submaximal exercise protocol with an exclusively female study population ($n=12$), although the participants were recreationally active and young (average age of 23 yrs.). Considering that both Wickham et al. (Wickham et al., 2019) and the present study found no difference in ExEc with BRS, sex differences should be considered as a possible explanation, at least in part, for the conflicting findings. However, it is likely that additional methodological differences between studies underlie the conflicting findings across investigations, as is discussed below.

The secondary aim of the present study was to determine the effects of BRS on resting and post-exercise BP in our study participants. Although there was no treatment difference at baseline or pre-exercise, post-exercise diastolic BP was significantly lower with BE compared to PL. This contrasts with that of Amaral and colleagues (2019), who investigated the effects of acute BRS on post-exercise BP in 13 female participants (average age 58 yrs.) and reported

no difference in post-exercise BP between treatments. In the present study, 7 of our participants were postmenopausal and 4 were perimenopausal, but were all healthy, trained individuals, whereas Amaral et al.'s participants were all post-menopausal, but were untrained and hypertensive. It is unclear why diastolic BP was reduced in our normotensive population but not in the hypertensive population in Amaral et al. (Amaral et al., 2019). It is important to study females who are peri-menopausal, menopausal, and post-menopausal, since menopause is associated with a 2-fold greater risk of hypertension (Barton Meyer, 2009); finding ways to reduce risk of developing hypertension and reducing overall cardiovascular disease risk is of great public health importance for this significant portion of the population.

Stanaway and colleagues (Stanaway et al., 2019) investigated differences in the BP response to BRS in older (age 56 yrs.) vs younger (age 25 yrs.) male and female adults, and found that while resting systolic and diastolic BP was decreased to the same extent in both age groups with BRS compared to placebo, the decrease in diastolic BP was significantly greater in the older compared to the younger group. In the present study, the treatment difference in systolic BP approached significance at $p = 0.053$ (treatment by time), while diastolic BP was significantly lowered. Taken together, the findings of Stanaway et al. (2019) and those of the present study suggest that BRS may benefit older individuals to a greater extent for blood pressure reduction purposes, particularly in lowering diastolic BP.

The methodological differences that likely underlie the conflicting findings among studies of BRS and exercise, BP, and ExEc include differences in the populations of interest and participant characteristics (e.g., sex, training status, and age), and the timing and frequency of supplement ingestion. However, perhaps the most important difference between studies of acute BRS is the NO_3^- content (i.e., dose) of the supplements given. Gallardo and Coggan (2018) suggested that the minimal effective NO_3^- dose to impact exercise efficiency is at least 5.0 mmol; they also analyzed the nitrate content of several commercially available BRS, and determined that the BRS used in the present study (BeetElite, Human^N Co., Austin, TX, USA) contained only 2.16 ± 0.28 mmol NO_3^- . A recent review by Macuh and Knep (2021) suggested that BRS is an effective ergogenic aid when taken in the dose range of 5 to 16.8 mmol, 2 to 3 hours before exercise. Lorenzo Calvo and colleagues (2020) reported a similar finding based on their system-

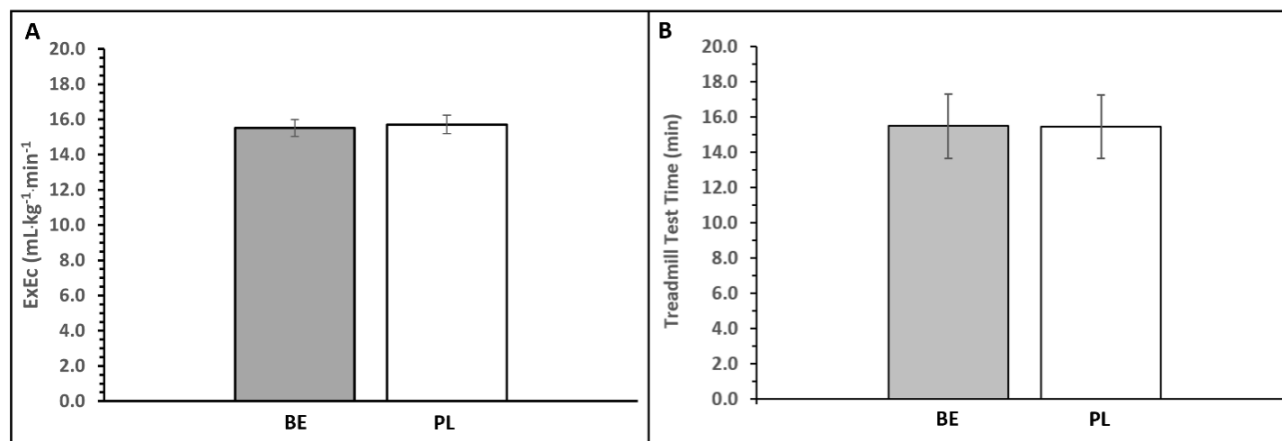


Figure 5: Treadmill test time and peak oxygen consumption (VO_2).

Note. (A) Total treadmill time; test was terminated, and the end time recorded, when participants reached 85% of age-predicted maximum heart rate. No difference was found between treatments ($p = 0.915$). (B) Peak VO_2 during the treadmill test for each treatment; no difference was found between treatments ($p = 0.314$). Values are mean \pm SE.

atic review, concluding that the effective range is 6 to 12.4 mmol taken 2 to 3 hours before exercise; however, they reported that the effects of BRS on ExEc are unclear, even when taken within the effective range. Therefore, our findings of no difference between BRS and placebo are likely due to the minimal NO_3^- content and timing of dosage. However, NO_3^- content differences do not solely explain differences in findings between studies. Wickham and colleagues (2019) found no differences in ExEc despite their BRS containing 26 mmol, and Amaral et al. (2019) reported no difference in post-exercise BP, despite administering a relatively high dosage BRS (20.78 mmol).

Another factor that may explain differences in findings is the timing of supplement administration. Most of the acute studies provide BRS 2 to 3 hours prior to exercise and/or resting BP measurement (Amaral et al., 2019; Lansley et al., 2011a; Muggerridge et al., 2013; Stanaway et al., 2019; Vanhatalo et al., 2010; Wickham et al., 2019). It has been reported that while plasma NO_3^- concentrations rise rapidly within 30 min of BRS ingestion and may peak as early as 1.5 h post-ingestion (Webb et al., 2008), the peak plasma concentration most likely occurs closer to 3 h after ingesting a BRS containing 4 mmol or greater NO_3^- (Kapil et al., 2010). Therefore, the timing of our dose in the present study likely contributed to the findings of no difference in ExEc.

There are limitations to the present study. First, it is possible that starting our treadmill protocol at 30-min post-supplementation may have been too soon to detect an effect on oxygen consumption. Second, we did not assess plasma nitrite

and nitrate levels, which would have given a more accurate picture of the plasma response to the treatments. Third, we used a treadmill exercise test in trained swimmers rather than a swimming test; however, this was done due to the significant challenges associated with collecting VO_2 data during a swimming test. Lastly, the dietary nitrate content of the chosen supplement may have simply been too low to elicit a physiological effect on oxygen consumption.

5 Conclusion

Acute beetroot supplementation did not improve exercise economy in this population of trained female masters athletes, although it did lower diastolic blood pressure in this healthy, normotensive group. Further research is warranted in menopausal and post-menopausal females, including those who are hypertensive, and in other female masters athlete groups.

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Conflict of Interest

The Human^N Co., Austin, TX, provided the beetroot supplement for the study.

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