Functional Sympatholysis and Blood Flow: Regulatory Changes with Duty Cycle, Sodium Intake, and Dietary Nitrate Supplementation

by

Jacob Troy Caldwell

B.S., Eastern Michigan University, 2011
M.S., Eastern Michigan University, 2015

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Department of Kinesiology
College of Human Ecology

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Abstract

During exercise, muscle blood flow ($\dot{Q}_m$) increases to match metabolic demand of the active skeletal muscle. In order for this matching to take place, 'competition' between local vasodilating metabolites and sympathetically mediated vasoconstriction, termed "functional sympatholysis," must take place. A key feature of functional sympatholysis is that it is driven largely by metabolic rate (i.e., a higher work rates lead to greater sympatholysis), but may also be largely dependent on nitric oxide bioavailability and oxidative stress in certain disease states (e.g., hypertension). Thus, evaluation of these factors may provide valuable insight into the vascular control mechanisms during exercise in both health and disease. Therefore, the purpose of this dissertation was to 1) determine the role metabolic rate and blood flow on mediating functional sympatholysis, 2) determine the role of nitric oxide bioavailability on functional sympatholysis with high salt intake, a risk factor for primary hypertension, and 3) determine the effect of increases in nitric oxide bioavailability on functional sympatholysis in primary hypertension patients.

In the first investigation (Chapter 1), we increased the relaxation phase of the contraction-relaxation cycle to increase active skeletal muscle blood flow ($\dot{Q}_m$) and see if this would impact vasoconstriction of the active skeletal muscle. We showed that a decreased relaxation time led to greater functional sympatholysis. Interestingly, despite a lower metabolic rate (15% and 20% MVC), we showed that there was no difference in vasoconstriction between the increased relaxation times. These results may show that increases in $\dot{Q}_m$ play a role in functional sympatholysis when mechanical compression is minimized. In the second investigation (Chapter 2), we sought to determine if high dietary sodium (HS) intake would impact functional sympatholysis. We showed that HS intake (15g/day for 7 days) did not impact functional sympatholysis during exercise. Importantly, we show a significant increase in mean arterial pressure (i.e., pressor response) during handgrip exercise. These findings show the deleterious changes in blood pressure, but further work is needed to pinpoint specific mechanisms causing the responses. In the final investigation (Chapter 3), we used an acute nitrate rich (NR) supplement to improve NO bioavailability in hypertensive post-menopausal women (PMW), and observe the impact on functional sympatholysis. We provide novel evidence that functional

sympatholysis is improved (~50%) with a NR supplement. The finding that a NR supplement can attenuate vasoconstriction in hypertensive PMW sheds light on the complexities of hypertension, functional sympatholysis and NO bioavailability.

The current results indicate that the ‘competition’ between vasodilating metabolites and sympathetically mediated vasoconstriction can be independently modified in health and disease. In individuals with impairment to local vasodilation (e.g., hypertension), the ability to increase functional sympatholysis and muscle blood flow may lead to improvements in cardiovascular health. Taken together, the present results suggest that modifying duty cycle, sodium intake, and NO bioavailability are important factors to be considered with regard to overall cardiovascular health.

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Acknowledgements

To all of you who worked with me in the laboratory over the past four years, thank you.

Dedication

This dissertation is dedicated to my grandmother, Marina L. Joseph, and my grandfather, Ronald G. Joseph (1942-2010). Thank you both for your wisdom, kindness, and occasional "tough love." This would not be possible without your patience and guidance.

Love, your grandson.

Preface

Chapters 2, 3 and 4 of this dissertation represent original research articles that are currently accepted or are in process to be sent to peer-review with the citations are listed below.

1. Caldwell JT, Sutterfield SL, Post HK, Lovoy GM, Hammer SM, Alexander AM, Barstow TJ, Ade CJ. Vasoconstrictor Responsiveness Through Alterations in Relaxation Time and Metabolic Rate during Rhythmic Handgrip Contractions (In press; Physiological Reports)

2. Caldwell, JT, Post, HK, Lovoy, GM, Sutterfield, SL, Banister, HR, Ade, CJ. Impact of High Sodium Intake on Blood Pressure and Functional Sympatholysis during Handgrip Exercise.

3. Caldwell JT, Sutterfield SL, Craig JC, Baumfauk DR, Copp CW, Ade CJ. Impact of Acute Dietary Nitrate Supplementation during Exercise in Hypertensive Women.

Chapter 1 - A Brief History of Functional Sympatholysis

The ability of the cardiovascular system to increase blood flow with concomitant increases in metabolic demand is essential for physical performance (1, 2). During the rest-to-exercise transition blood flow is redistributed to the active skeletal muscle by the processes of vasoconstriction and vasodilation (2). Specifically, an increase in efferent outflow to the periphery increases norepinephrine release, which acts on the α_1 and α_2 adrenergic receptors on the vascular smooth muscle to cause vasoconstriction. However, in healthy individuals it is well known that increases in exercise intensity (and sympathetic outflow) do not impair limb blood flow in the exercising limb. One concept, coined "functional sympatholysis," that explains this suggests that for a given increase in α_1 and α_2 mediated adrenergic vasoconstriction within the contracting limb, a concurrent, metabolically driven, increase in muscle metabolites attenuates the vasoconstriction (3-9). This concept highlights an area still under active investigation and continues today to be a fundamental control mechanism in blood flow regulation during exercise (10-12).

The first published report of functional sympatholysis appeared in 1962 by Remensnyder et al. (1962). In this investigation they used a canine hind-limb model with electrical stimulation to evoke muscular contraction. Importantly, they observed a diminished vasoconstriction to increased sympathetic activity during active muscular contraction. In their study, the pressure/flow curves indicated in figure 10 of their manuscript clearly show from rest to rest + carotid occlusion, a substantial increase in vasoconstriction, shown by an upward and leftward shift in mean pressure and blood flow, respectively. However, during hind-limb contraction, these conditions appeared to completely attenuate the increase in sympathetic outflow during carotid occlusion as no shift in the curve was present (3).

The next major advancement in functional sympatholysis came roughly 30 years later (4, 13). Thomas et al. (1994) found that functional sympatholysis, in Sprague-Dawley rats, was apparent in glycolytic, but not oxidative fibers. Further, they showed that α_2, but not α_1, adrenergic receptors were attenuated during exercise. They did this by

electrically stimulating the lumbar sympathetic nerves to increase sympathetic nerve activity at rest and during maximal tetanic contractions in the rat hindlimb. Specifically, to mechanistically determine which adrenergic receptor was responsible, selective adrenergic agonists (e.g., norepinephrine – α_1/α_2, phenylephrine – α_1, and UK-14304 – α_2) were used. This study showed that phenylephrine evoked a decrease in femoral vascular conductance during contractions; however, during lumbar stimulation, norepinephrine, and UK-14304 contractions attenuated the decrease in femoral vascular conductance. This investigation marked the beginning of the mechanistic underpinnings that may drive functional sympatholysis.

While the data in animal models showed that exercise attenuates α_2, but not α_1 adrenergic receptors, Rosenmeier et al. (2003) indicated that both α_1 and α_2 adrenergic receptors were attenuated in the exercising forearm of humans. With the use of tyramine (α_1/α_2 agonist), phenylephrine (α_1 agonist) and clonidine (α_2 agonist), they provided the first evidence that forearm exercise attenuated both α_1 and α_2 adrenergic responses (14). Based on the aforementioned investigations, functional sympatholysis is now a well-accepted phenomena; however, the next hurdle was in figuring out which metabolite(s), produced during exercise, actually attenuated the α-adrenergic mediated vasoconstriction (7, 13-20).

The first metabolites proposed to attenuate α-adrenergic vasoconstriction were hydrogen ions, prostaglandins, nitric oxide (NO), adenosine-triphosphate, tissue hypoxia, and potassium channels (19). However, recent evidence suggests that NO and ATP are the key metabolites produced within the contracting limb that mediate functional sympatholysis (10, 20-22). Studies have either increased adrenergic agonists like noted above (9, 15, 23), or have stimulated endothelial-dependent vasodilators (e.g., NO, acetylcholine, adenosine) (20, 21). Interestingly, a certain degree of contention exists between animal and human investigations. As expected, further work is needed to clarify the issue of which, if any, specific metabolite can effectively attenuate α-adrenergic vasoconstriction. The following chapters highlight the complexities and methods that may also impact functional sympatholysis and the regulation of blood flow.

Chapter 2 - Vasoconstrictor Responsiveness Through Alterations in Relaxation Time and Metabolic Rate During Rhythmic Handgrip Contractions

Jacob T. Caldwell, Shelbi L. Sutterfield, Hunter K. Post, Garrett M. Lovoy, Heather R. Banister, Shane M. Hammer, Carl J. Ade

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Summary

Increasing the relaxation phase of the contraction-relaxation cycle will increase active skeletal muscle blood flow ($\dot{Q}_m$). However, it remains unknown if this increase in $\dot{Q}_m$ alters the vasoconstriction responses in active skeletal muscle. This investigation determined if decreasing mechanical impedance would impact vasoconstriction of the active skeletal muscle.

8 healthy men performed rhythmic hand-grip exercise under three different conditions; 'low' duty cycle at 20% maximal voluntary contraction (MVC), 'low' duty cycle at 15% MVC, and 'high' duty cycle at 20% MVC. Relaxation time between low and high duty cycles were 2.4 s vs. 1.5 s respectively. During steady-state exercise lower body negative pressure (LBNP) was used to evoke vasoconstriction. Finger photoplethysmography and Doppler ultrasound derived diameters and velocities were used to measure blood pressure, forearm blood flow (FBF: $ml \cdot min^{-1}$) and forearm vascular conductance (FVC: $ml \cdot min^{-1} \cdot mmHg$) throughout testing.

The low duty cycle increased FBF and FVC versus the high duty cycle under steady-state conditions at 20%MVC ($p<0.01$). The high duty cycle had the greatest attenuation in %ΔFVC (-1.9±3.8%). The low duty cycle at 20% (-13.3±1.4%) and 15% MVC (-13.1±2.5%) had significantly greater vasoconstriction than the high duty cycle (both: $p<0.01$) but were not different from one another ($p=0.99$). When matched for work rate and metabolic rate ($\dot{V}O_2$), the high duty cycle had greater functional sympatholysis than the low duty cycle. However, despite a lower $\dot{V}O_2$, there was no difference in functional sympatholysis between the low duty cycle conditions. This may suggest that increases in $\dot{Q}_m$ play a role in functional sympatholysis when mechanical compression is minimized.

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Introduction

During steady-state rhythmic exercise, the majority of muscle blood flow ($\dot{Q}_m$) is delivered during the relaxation phase of the contraction-relaxation cycle (i.e., duty cycle) (24, 25). Thus, increasing duty cycle (i.e., increasing mechanical impedance) may decrease $\dot{Q}_m$ due to a shorter relaxation phase (26). As such, increasing duty cycle has been shown to reduce the capacity to sustain a set power output during hand-grip exercise (24, 25). This is thought to be attributed to increases in mechanical impedance, which will result in increased muscle fatigue and compromised exercise capacity (24, 25). Conversely, lowering the duty cycle for a given contraction frequency will result in less mechanical impedance and increased oxygen delivery (24). Thus, the lower duty cycle will increase $\dot{Q}_m$, consequently leading to higher sustainable aerobic power outputs (25).

While it is known that the increased relaxation period facilitates greater O_2 delivery to the active skeletal muscle, it remains unclear if decreasing the mechanical impedance (increased relaxation), can impact sympathetically mediated vasoconstriction (i.e., functional sympatholysis) (3, 4, 27). One may speculate that decreasing mechanical impedance will impact functional sympatholysis in active skeletal muscle via changes in the internal milieu of the contracting muscle (i.e., decreased metabolite production and accumulation) (4, 28, 29). Recent work by Kruse at al. (2017) demonstrated that slower contraction-relaxation frequencies (1:2 s) were less likely to maintain $\dot{Q}_m$ when sympathetic outflow was increased. However, their use of a slower contraction frequency vs. duty cycle not only increased relaxation time, but decreased metabolic rate as well, which would also impact vasoconstriction if less glycolytic fibers were recruited (4). An example of altering relaxation time with matched metabolic rate is best shown by Bentley et al. (2017), in that they used an inflatable cuff to lengthen mechanical impedance of the duty cycle, reducing relaxation time and $\dot{Q}_m$. They showed a greater rebound in $\dot{Q}_m$ during the higher duty cycle (i.e., shorter relaxation) versus the control condition to offset the increase in mechanical impedance. The rebound in $\dot{Q}_m$ during the reduced relaxation phase indicated a compensatory vasodilation to support greater $\dot{Q}_m$ and suggests that metabolite buildup may play a key role in metabolic vasodilation (24). As such, altering duty cycle without changes to

contraction frequency may provide additional insight on mechanical impedance and functional sympatholysis (30).

To the best of our knowledge, there are no current reports that have examined if increasing the relaxation phase during hand grip exercise alters functional sympatholysis during lower body negative pressure (LBNP) evoked vasoconstriction. Therefore, the purpose of the current investigation was to test the hypotheses that a low duty cycle (i.e., decreased mechanical impedance) would elicit a larger vasoconstriction during increased sympathetic outflow compared to a high duty cycle (i.e., increased mechanical impedance) at a matched work rate. Further, given that the majority of evidence has demonstrated that metabolic rate dictates functional sympatholysis, we also hypothesized that a lower work rate with increased relaxation time would elicit a larger vasoconstrictor response than the matched duty cycle. From this information we can begin to investigate the relationship between in duty cycle and vasoconstriction responses.

Methods

Participants

Eight healthy, recreationally active, men [age 25 ± 2 years (mean ± SE); height 177 ± 1 cm; mass 84 ± 5 kg] volunteered to participate in the current investigation. All participants reported to the laboratory after a minimum 3-hour fast and were asked to avoid heavy exercise for 24 hours or caffeine and alcohol prior to data collection. Based on a physical activity questionnaire, all participants completed less than 5 total hours of recreational activity per week. All experimental procedures and methods were approved by the Institutional Review Board of Kansas State University and conformed to the standards set forth by the Declaration of Helsinki. Prior to data collection all subjects signed an informed consent and filled out a health history screening form for overt diseases (e.g., cardiovascular, metabolic, renal). All testing was completed in a temperature-controlled laboratory (20-22 °C) at the same time of day.

Experimental Measurements

Beat-by-beat mean arterial pressure (MAP) was measured via finger photoplethysmography (Finometer Pro, FMS, The Netherlands) and calibrated to brachial artery blood pressure according to manufacturer specifications. Measurements of brachial artery diameter and blood velocity were simultaneously measured with an ultrasound system (LOGIQ S8, GE medical systems, Milwaukee, WI) equipped with a multi-frequency linear array transducer operating at 10 MHz and placed ~10 cm proximal to the antecubital fossa with care taken to avoid the bifurcation of the artery. All measurements had a Doppler sample volume set at the full width of the vessel with the insonation angle <60° and were captured during both rest and exercise. Brachial artery images were stored offline and diameters were analyzed using a commercially available edge-detection and wall-tracking software package (Vascular research tools 6, [Medical Imaging Applications, Coraville, Iowa]) as described previously (31).

Near-Infrared Spectroscopy (NIRS)

Microvascular heme concentrations (i.e., hemoglobin + myoglobin) were measured with a frequency domain multi-distance NIRS probe (OxiplexTS, ISS, Champaign, IL, USA) that was placed longitudinally over the flexor digitorum superficialis of the exercising arm and had a

black cloth placed over the site to limit light, described in detail previously (32). Briefly, the NIRS probe consists of a detector fiber bundle, four light-emitting diodes (LED), and operates at wavelengths of 690 and 830 nm (source-detector distance 2.5 – 4.0 cm). The NIRS device allows for absolute (μM) quantification of total-[heme] and deoxygenated heme concentration (deoxy-[heme]). Importantly, because the NIRS device cannot dissociate myoglobin from hemoglobin, the term [heme] is used herein. The NIRS probe was calibrated prior to each test using a phantom block supplied by the manufacturer. Identification of the muscle belly was identified by a single experienced investigator palpating during muscle contraction and remained in position throughout testing. The NIRS data were collected throughout the protocol at 50Hz, stored for post hoc analysis, and time aligned with the blood pressure and blood flow data.

Lower Body Negative Pressure

Subjects were placed into a custom-built LBNP chamber in a supine position. Once subjects were in the chamber, an initial test to familiarize and confirm a proper seal of the chamber was performed. The level of LBNP (~30 mmHg) used in the current investigation has been confirmed previously to primarily unload the cardiopulmonary baroreceptors without altering arterial pressure and provides reproducible increases in muscle sympathetic nerve activity in the forearm (33-35). LBNP was used at rest and during steady-state exercise for 2-minutes to allow resting and exercising vasoconstriction comparisons.

Experimental Protocol

Testing was performed while subjects were supine in a custom-built LBNP chamber based on previously reported specifications (36). All exercise was performed by dynamically contracting a custom-built two-pillar hand-grip dynamometer with a maximal displacement of 2.5 cm. Maximal voluntary contraction (MVC) was calculated by taking the average of the two highest (three total trials with one minute between each) MVCs and was used to calculate 20% MVC. Subjects underwent a randomized-crossover design and performed two exercise bouts at either 20% (low) or 50% (high) duty cycle, described previously (25). Briefly, the low duty cycle matches the high duty cycle's concentric contraction time (0.6 s) (37, 38). However, the low duty cycle excludes the isometric transition phase (0.3s), and the eccentric relaxation phase (0.6s) (Figure 1.1). To best control for potential differences in metabolic rate across each duty cycle,

work rate (20% MVC) and contraction frequency (20 contractions·min^{-1}) were kept constant. After preliminary data was analyzed a second experimental day was performed to determine the impact of relaxation time at a different metabolic rate, by performing the experiment at 15% MVC with the low duty cycle. The results of resting data for day two showed an identical vasoconstrictor response and was excluded from analysis to reduce redundancy of results.

Prior to the initiation of hand-grip exercise, a 2-minute baseline coupled with 2-minutes of LBNP (~ 30 mmHg) were completed to establish a 'resting' vasoconstrictor response (i.e., no functional sympatholysis). Next, a 2-minute baseline and 7-minute hand-grip protocol was performed. During the final two minutes of hand-grip exercise LBNP was used to observe changes in the 'exercising' vasoconstrictor response (i.e., functional sympatholysis). After the first bout of hand-grip exercise, a minimum of 10 minutes recovery was given to allow forearm blood flow (FBF) and blood pressure to return back to steady baseline values. This was confirmed by similar brachial artery velocity profiles and the hand-grip protocol was performed again.

Data Analysis

All data were time aligned and averaged into 1-minute bins during resting and steady-state exercise measurements. FBF was calculated as: FBF = mean blood velocity • 60 • π • (brachial diameter/2)^2 calculated in ml · min^{-1}. Importantly, MAP was time aligned with FBF to calculate forearm vascular conductance (FVC) calculated in ml · min^{-1} · 100 mmHg [FVC = (FBF / MAP) * 100]. At rest, FBF, FVC, and MAP measurements were averaged across the second minute (min 1-2) of rest and LBNP (min 3-4). During exercise, FBF, FVC, and MAP were calculated as a minute average prior to LBNP (steady-state: min 4-5) and during the final minute of LBNP (min 6-7). Functional sympatholysis was calculated as: $\%\Delta FVC = FVC_{LBNP} - FVC_{ss})/ FVC_{ss} \times 100$, where 'ss' denotes steady-state (7, 39). Following data collection, NIRS data were averaged into 1-min epochs for deoxy-[heme] and total-[heme], which were time aligned with blood flow and blood pressure. In addition, Craig et al. 2017 used an adjustment to the NIRS output to adequately return signal values to original [Heme] concentrations and the NIRS signals were multiplied by 4. The original syntax used for the NIRS was based on brain oxygenation (key chromophore is Hb) and the signal was divided by 4 in the software. It is now

known that [Mb] plays a significant role in the skeletal muscle oxygenation (40) and must have this correction applied. The new concentration agrees with previous data of muscle biopsy [Mb] when transformed into appropriate units (41).

Estimation of Forearm Oxygen Consumption

To quantify forearm metabolic rate, $\dot{V}O_2$ was calculated as a function of brachial artery blood flow and deoxy-[heme], a proxy for arterial-venous O_2 difference (42, 43), as described previously (25, 40). The deoxy-[heme] values are in μmol heme/l tissue, and tissue is assumed to be metabolically active skeletal muscle. The deoxy-[heme] values can be converted into μmol heme/l blood using a conversion of 1.36% capillary blood volume/muscle volume (taken from 400 cap/mm^2, 28.2 μm^2 cross sectional area, and a coefficient of 1.2 which corrects for the tortuosity and branching of the capillaries) (44). These units can then be converted using specific units (mole O_2/L blood) assuming that 1 mole O_2/mole heme, and further to LO_2/L blood using the conversion 22.4L O_2 /mole O_2. $\dot{V}O_2$ values in LO_2/min may then be obtained by simply multiplying this value by the measured brachial artery blood flow.

Statistics

Data were analyzed with commercially available statistical software package (Sigmaplot; version 12.5, Systat software, San Jose). MAP, FBF, FVC, deoxy-[heme], total-[heme], and $\dot{V}O_2$ were analyzed with a two-way repeated measures ANOVA with a Bonferroni correction for pairwise comparison. The level of significance was set at ($p < 0.05$). All data were presented as means ± standard error.

Results

Steady-state exercise hemodynamic responses

Significant interactions (time x condition) were present for FBF and FVC, and $\dot{V}O_2$ ($p < 0.01$), but not MAP ($p = 0.07$) or NIRS derived variables (all: $p = 0.10$). Pairwise tests revealed that FBF and FVC during the low duty cycle at 20% MVC were significantly greater than the high duty cycle (20% MVC) and low duty cycle (15% MVC) (Table 1.1; $p < 0.01$). The integration of FBF and deoxy-[heme] yielded similar steady-state $\dot{V}O_2$ response during the low and high duty cycles at 20% MVC (Table 2.1, $p > 0.05$). Importantly, the low duty cycle at 15% MVC had significantly lower $\dot{V}O_2$ compared to both duty cycles at 20% MVC ($p = 0.02$). Deoxy- and total-[heme] NIRS variables were not significantly different between duty cycles (table 2.1; $p > 0.05$).

Steady-state exercise + LBNP hemodynamic responses

Pairwise tests revealed a significant reduction in resting FBF and FVC with the application of LBNP (both; $p < 0.01$). During exercise, FBF and FVC were significantly decreased with LBNP during both low duty cycle conditions ($p < 0.01$: 20%MVC; $p = 0.03$: 15%MVC). However, there was no significant decrease in FVC or FBF during the high duty cycle (both: $p > 0.05$). Deoxy- and total-[heme] NIRS variables were not significantly different between duty cycles during LBNP (table 2.1; $p > 0.05$).

Figure 2.1 illustrates the calculated functional sympatholysis response for each condition. The %ΔFVC was significantly attenuated by exercise in all conditions ($p < 0.01$). The %ΔFVC during both low duty cycle was significantly lower than the high duty cycle (Figure 2.1; $p = 0.01$; $p = 0.03$, respectively). There was no significant difference between %ΔFVC during the low duty cycles ($p = 0.99$). As previously mentioned in the methods, data from day two was not analyzed due to identical responses to day one (day 1: %ΔFVC: -33.36%; day 2: %ΔFVC: -34.33%).

Discussion

The major new finding of the current investigation is that decreasing mechanical impedance, which allowed for an increased relaxation time, altered vasoconstriction responses in the active skeletal muscle. We show an ~10% greater vasoconstrictor response during the low duty cycles compared to the high duty cycle, suggesting that the high duty cycle better attenuated LBNP evoked vasoconstriction. Interestingly, at a matched duty cycle, but different metabolic rate (i.e., 15% and 20% MVC), we demonstrate that functional sympatholysis was not significantly different (Figure 2.1). These findings may suggest that when mechanical compression is minimized, increases in $\dot{Q}_m$ play a role in functional sympatholysis.

In the current investigation, we demonstrate that a low duty cycle shifts the balance between vasoconstriction and vasodilation of the active skeletal muscle to a more pronounced vasoconstriction during LBNP (Figure 2.1). We have demonstrated, during 20 % MVC, that there is an ~10% larger vasoconstriction when mechanical impedance is decreased. Interestingly, even with the greater vasoconstriction, the low duty cycle was shown to have a bulk blood flow that was ~22 ml/min above the high duty cycle; however, this finding did not reach statistical significance (Table 1.1). Further, it may be that the higher blood flow prior to LBNP was above that needed to maintain metabolic demand and a significant vasoconstriction was negligible. In a previous investigation it was shown, with intravital microscopy, that low blood flows better attenuate norepinephrine mediated vasoconstriction (45). As such, our current findings suggest that increased $\dot{Q}_m$ increased LBNP evoked vasoconstriction. However, the current study is limited in speculating on specific mechanism(s) influencing the vasoconstrictor response, but may be linked to increased metabolite clearance and/or improvements in oxidative metabolism (45-47).

During 20% MVC, functional sympatholysis was greater during the high duty cycle compared to the low duty cycle (Figure 2.1). This agrees with our hypothesis and raises another important point; when work rate (20% MVC), and contraction frequency (20/min) are matched, differences in mechanical impedance and time under tension likely influenced the results (24, 48). For example, Bentley et al. (2017), showed that greater mechanical impedance, like that

shown with the high duty cycle, will briefly impair blood flow resulting in a "rebound" vasodilation to acutely increase $\dot{Q}_m$. As such, it is likely that mechanical impedance influenced this rebound effect during the relaxation phase, potentially influencing functional sympatholysis. In theory, the increased mechanical impedance may have augmented the vasoactive metabolites, and functional sympatholysis, within the active musculature (48). Yet, muscle metabolites were not measured within the present investigation and further interpretation is limited.

Contrary to our hypothesis, the low duty cycle at 15% MVC did not show an increase in LBNP evoked vasoconstriction when compared to the low duty cycle at 20% MVC condition. This is an interesting point given that the low duty cycle at 15% MVC had a significantly lower VO2 compared to the low duty cycle at 20% MVC. This data suggests that metabolic rate can differ and still show a similar LBNP evoked vasoconstriction responses. While the majority of evidence suggests that functional sympatholysis largely is driven by metabolic rate (7, 28, 29, 39), we provide the first evidence that duty cycle caused a similar degree of vasoconstriction during different metabolic rates. It is unknown why our data go against this previous work, but may be due to the similar microvascular tissue oxygenation (i.e., deoxy-[heme], total-[heme]), as determined by NIRS, between the conditions, suggesting that in our experimental set-up there was an adequate delivery of muscle blood flow for the given metabolic demand (49).

A strength of the current investigation was the maintenance of contraction frequency (20 contractions/min), duration (3 s), and intensity (20% MVC) during dynamic hand grip exercise. This led to similar estimates of $\dot{V}O_2$ between both duty cycles at 20% MVC (Table 2.1), and a lower $\dot{V}O_2$ at 15% MVC. This is important given that Kruse et al. (2017) demonstrated that vasoconstrictor responses were independent of contractile 'work' and dependent on metabolic rate by altering contraction frequency (20 contractions/min versus 10 contractions/min). Our results extend that of Kruse et al. (2017) by demonstrating that duty cycle and the subsequent changes in $\dot{Q}_m$, may also directly alter functional sympatholysis. Importantly, we show that the low duty cycles had a similar level vasoconstriction at different metabolic rates. As mentioned above, it may be that a relative overperfusion allowed a certain degree of vasoconstriction to take place, but more work in this area is needed. Taken together, in populations that are blood flow limited, lowering the duty cycle may be a viable option to improve $\dot{Q}_m$ (50).

Experimental considerations

The current investigation used LBNP to increase sympathetic outflow to the periphery. As such, it cannot be determined if pre-or-post junctional adrenergic receptors were mediating the responses found in the current study. Second, $\dot{Q}_m$, and deoxy-[heme] from the NIRS were used to calculate $\dot{V}O_2$ during handgrip exercise with the description of this discussed in the methods. As such, the accuracy of $\dot{V}O_2$ may be hindered by assumptions used but were held constant to limit influence of the assumptions on detected changes found (2). Two assumptions were made to calculate $\dot{V}O_2$ with NIRS: 1) it was assumed that the deoxy-[heme] signal is a reflection of only hemoglobin; however, it is known myoglobin plays a significant role (40); and 2) it was also assumed that the entire NIRS signal came from muscle without any impact of adipose tissue in the forearm. Moreover, the values obtained for $\dot{V}O_2$ in the current study are similar to the direct measurements shown previously (51). Finally, the metabolic rate ($\dot{V}O_2$) was influenced by the different time under tensions when comparing between each duty cycle. This would directly impact the ATP cost of contraction and must be considered with regard to the findings presented.

Conclusion

The current investigation has demonstrated that the ability to attenuate sympathetically mediated vasoconstriction (i.e., functional sympatholysis), during muscular contraction may shift toward an increased vasoconstriction if the contraction-relaxation cycle (i.e., duty cycle) is manipulated to lower mechanical impedance and increase $\dot{Q}_m$. Moreover, it was interesting to note that a low duty cycle at 15% MVC did not elicit a larger vasoconstriction relative to the 20% MVC condition, suggesting adequate delivery of muscle blood flow for the given metabolic demand. This investigation highlights the potential for utilizing a lower duty cycle to improve bulk $\dot{Q}_m$ in individuals with compromised transport. This may allow individuals to sustain a higher exercise intensity and/or increase exercise tolerance.

Table 1.1 Hemodynamic response

	Forearm blood flow (ml min^{-1})			Mean arterial pressure (mmHg)			Forearm vascular conductance (ml min^{-1} 100 mmHg)		
Resting	84	±	11*	88	±	3	97	±	13*
Resting + LBNP	51	±	5 *†	88	±	2	59	±	6 *†
Low duty cycle (20% MVC)									
Steady-state	392	±	20* ‡	100	±	4	398	±	34 *‡
Steady-state + LBNP	344	±	26 *†	100	±	4	344	±	26 *†
High duty cycle (20% MVC)									
Steady-state	330	±	27*	103	±	5	327	±	37*
Steady-state + LBNP	322	±	18*	102	±	4	320	±	26*
Low duty cycle (15% MVC)									
Steady-state	282	±	13 *#	95	±	3	301	±	16 *#
Steady-state + LBNP	250	±	10 *†#	96	±	3	261	±	12 *†#

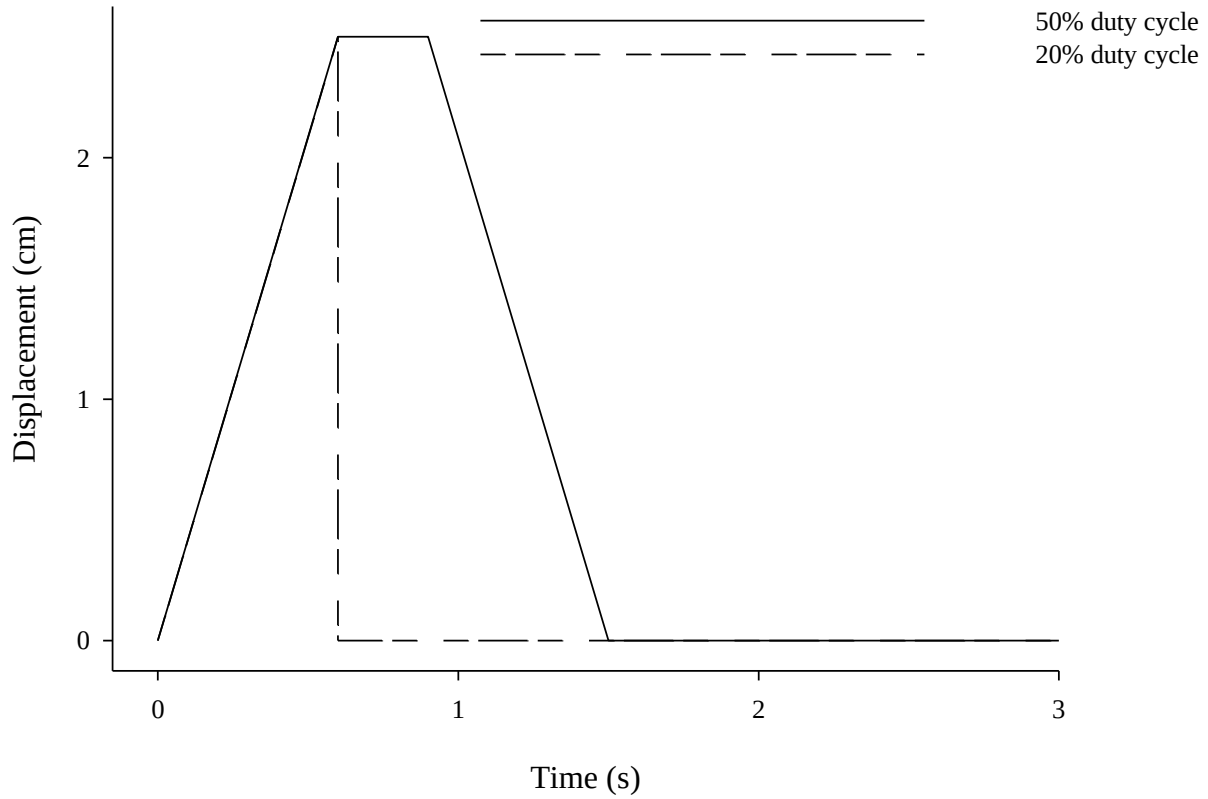
Hemodynamic response: forearm blood flow (FBF), mean arterial pressure (MAP), and vascular conductance (FVC) at rest and during exercise. * denotes significant main effect for time ($p < 0.01$). † denotes main effect of LBNP ($p < 0.01$). ‡ denotes significant difference from the high duty cycle in same condition ($p < 0.01$). # significant difference from low duty cycle (20% MVC) in same condition ($p < 0.01$). Data are means ± standard error.

Table 2.1 Metabolic response

	VO2 (ml min^{-1})			Deoxy-[heme] (μM)			Total-[heme] (μM)		
Resting	15.4	±	5.3*	83.6	±	5.2	338.0	±	18.8
LBNP	11.5	±	3.7*	98.8	±	3.2	322.8	±	18.0
Low duty cycle (20% MVC)									
Steady-state	71.6	±	6.7 *	113.6	±	8.0	387.6	±	24.8
Steady-state + LBNP	65.6	±	7.2 *	121.6	±	9.6	382.0	±	23.6
High duty cycle (20% MVC)									
Steady-state	67.6	±	5.8 *	126.0	±	10.8	367.2	±	19.6
Steady-state + LBNP	68.1	±	4.1 *	130.0	±	10	363.6	±	19.6
Low duty cycle (15% MVC)									
Steady-state	49.1	±	7.9 *†	100.4	±	9.2	354.0	±	20.8
Steady-state + LBNP	43.4	±	6.9 *†	100.4	±	9.6	352.4	±	20.4

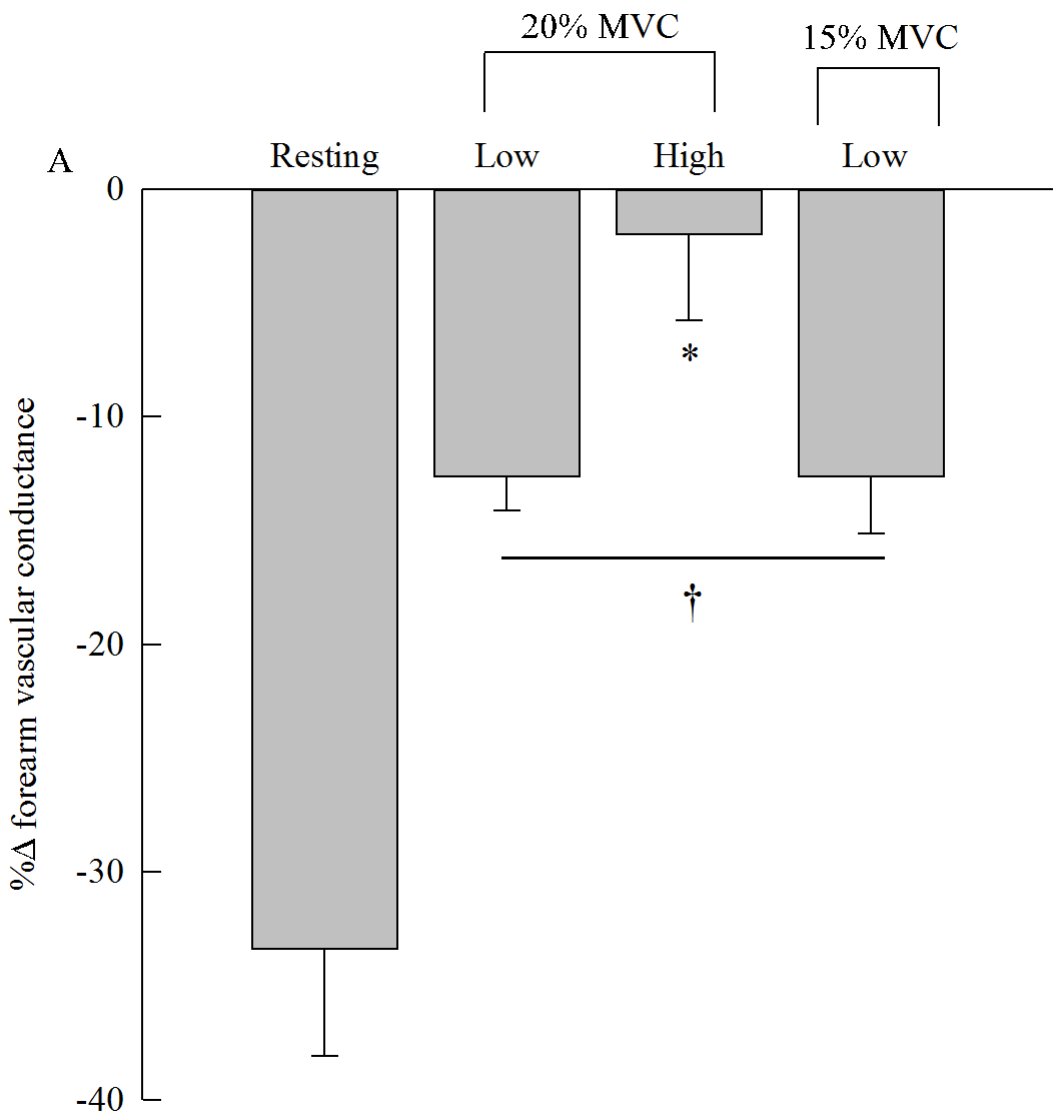
$\dot{V}O_2$, deoxy-[heme], and total-[heme] derived from NIRS ($\dot{V}O_2$) was the product of muscle blood flow and deoxy-[heme] (25). * denotes significant main effect for time ($p < 0.01$). † denotes significantly lower than 20% MVC conditions ($p < 0.01$). Data are means ± standard error.

Figure 1.1 Duty cycle paradigm



The low duty cycle (20%) matches the high duty cycle (50%) concentric contraction time (0.6 s) and excludes the isometric transition phase (0.3s), and the eccentric relaxation phase (0.6s).

Figure 2.1 Percent change in forearm vascular conductance



* denotes significant difference from low duty cycles ($p < 0.01$). † denotes significantly different from rest ($p < 0.01$). Data are means ± standard error. Low designates 20% duty cycle, high designates 50% duty cycle.

Chapter 3 - Impact of High Sodium Intake on Blood Pressure and Functional Sympatholysis During Handgrip Exercise

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Summary

High dietary salt intake is associated with an increased risk for the development of hypertension, cardiovascular (CVD) and cerebrovascular disease. Additionally, exaggerated blood pressure during dynamic exercise is independently predictive of future CVD beyond resting blood pressure and other conventional risk factors. This is critical given that in animal models, dietary salt negatively augments blood pressure responses during static hind-limb contractions, suggesting an increased risk of an adverse CVD event during exercise with high dietary salt. However, it currently remains unknown if high salt intake increases arterial blood pressure and impairs vascular function during voluntary dynamic exercise in healthy men and women. Based on previous preclinical reports, we hypothesized that high salt intake would decrease resting vascular endothelial function, increase arterial blood pressure during dynamic exercise and decrease functional sympatholysis. It was hypothesized that HS intake would increase the blood pressure response during rhythmic handgrip exercise and that functional sympatholysis would be attenuated. Thirteen healthy men and women underwent a randomized, double-blind, placebo-controlled trial for 7 days with 15g/day sodium chloride supplement or cellulose. Beat-by-beat blood pressure (BP) and heart rate were recorded throughout the trial on the non-exercising limb. Forearm blood flow (FBF) was calculated via mean blood velocity derived from ultrasonography on the brachial artery of the exercising limb. All patients performed a flow-mediated dilation protocol followed by (20% maximal voluntary contraction) submaximal hand-grip exercise for 7 min with lower-body negative pressure initiated during min 5 – 7. Normalized brachial artery flow-mediated dilation was significantly reduced during the HS condition (HS: $2.2\pm0.26^{e-5}$; Pl: $4.1\pm0.56^{e-5}$; $p<0.01$). Mean arterial BP was significantly higher at all time points compared to the placebo condition ($p<0.05$). However, functional sympatholysis was not different between conditions ($p>0.05$). In summary, the results of this study show the effects of HS intake on arterial blood pressure during handgrip exercise. These findings highlight that the augmented exercise blood pressures may be key mediator for the increased risk for adverse CVD outcomes associated with high dietary salt intake.

Introduction

A well-known modifiable risk factor in the development of cardiovascular disease (CVD) is high dietary salt intake (52). Yet, current data suggests that individual sodium intake exceeds established recommendations (53, 54). In individuals that are "salt-sensitive", high sodium (HS) intake is known to decrease endothelial-dependent vasodilation, increase arterial stiffness and cause hypertension; events that may precede the development of atherosclerosis (52, 55, 56). Increasing evidence also suggests that HS intake in individuals who are relatively "salt-resistant" show attenuated vascular control that is independent from changes in blood pressure (55, 56). In a recent report it was shown that HS intake augmented the exercise pressor reflex in Sprague-Dawley rats during static hind-limb contractions (57). The exercise pressor reflex is located in contracting skeletal muscle and is a feedback mechanism comprised of both mechanical (type III) and metabolic (type IV) afferent fibers (58). To date, only two investigations, both in animal models, have used HS intake during exercise to observe changes in blood pressure that was due to an increased exercise pressor reflex (57, 59). The majority of investigations in humans have only utilized resting hyperemic measurements (e.g., flow-mediated dilation (60)) or drugs (e.g., acetylcholine (61)) to investigate blood pressure/flow responses during HS intake. Thus, substantially less is known about the exercise pressor response to dynamic handgrip exercise with HS intake.

A key regulator of arterial pressure during exercise exists in the endothelial cells and their vasodilatory effect on vascular smooth muscle; however, endothelial dysfunction promotes inflammation and vasoconstriction and is considered a key step in the development of atherosclerosis and CVD (62). HS intake has been consistently shown in human investigations to reduce endothelial-dependent flow-mediated vasodilation (63, 64). Tzemos, Lim (61) have shown that 5 days of salt loading lowered endothelial-dependent increases in blood flow; yet, endothelial-independent vasodilator function remained normal. A proposed mechanism behind the decrease in vasodilation is decreased nitric oxide (NO) bioavailability coupled with lower endothelial NO synthase activity (eNOS) due, in part, to increases in oxidative stress (56, 65, 66). In addition, decreases in NO bioavailability have been shown to attenuate functional sympatholysis, which in turn creates an exaggerated vasoconstriction and decreases blood flow

during exercise (i.e., impaired functional sympatholysis) (19, 67, 68). Thus, it can be speculated that individuals consuming large amounts of dietary salt may cause an exaggerated vasoconstriction and influence blood pressure during exercise. It remains unknown if functional sympatholysis is attenuated in healthy individuals consuming high amounts of dietary salt. Based on this information, the aim of the current investigation was to delineate the impact of HS intake on the cardiovascular responses to rhythmic handgrip exercise. It was hypothesized: 1) that acute HS intake would increase the blood pressure response to exercise and 2): that functional sympatholysis would be attenuated during the HS condition.

Methods

Participants

Thirteen healthy, recreationally active, men (5) and women (7) [age 33 ± 5 years (mean ± SE); height 160 ± 2 cm; HS mass 79 ± 3, placebo mass 78 ± 2) volunteered to participate. The current investigation was a two-day randomized, double blind, placebo-controlled crossover study design. All participants reported to the laboratory after a 7-day sodium or placebo loading protocol and an overnight fast (~8 hours). Two of the female subjects only completed 5 days of sodium loading but were still included in all analyses. All participants were asked to avoid heavy exercise or caffeine 24 hours prior to data collection. All experimental procedures and methods were approved by the Institutional Review Board of Kansas State University and conformed to the standards set forth by the Declaration of Helsinki. Prior to data collection all subjects signed an informed consent and filled out a health history screening form for overt diseases (e.g., cardiovascular, metabolic, renal). All testing was completed in a temperature-controlled laboratory (20-25 °C).

Experimental Measurements

Beat-by-beat mean arterial (MAP), systolic (SBP), and diastolic blood pressures (DBP), and heart rate (HR) were measured via finger photoplethysmography (Finometer Pro, FMS, The Netherlands), calibrated to the brachial artery blood pressure according to manufacturer specifications. The Modelflow method was used to calculate relative changes in stroke volume (SV) and cardiac output ($\dot{Q}$) (69, 70). Systemic vascular resistance was calculated as MAP divided by $\dot{Q}$. Rate pressure product (i.e., work of the heart) was calculated as SBP x HR.

Endothelial-dependent Flow-mediated Dilation (FMD)

Brachial artery endothelium-dependent FMD was performed according to previously established guidelines after a ~10 min resting period (71). Briefly, participants had a 6 cm tourniquet blood pressure cuff (Hokanson SC5, Bellevue, WA, USA) connected to a rapid cuff inflator (Hokanson E20, Bellevue, WA, USA) placed just proximal to the antecubital fossa. Measurements of brachial artery diameter and blood velocity were simultaneously measured with an ultrasound system (LOGIQ S8, GE medical systems, Milwaukee, WI) equipped with a

multi-frequency linear array transducer operating at 10 mHz. All measurements had a Doppler sample volume set at the full width of the vessel with the insonation angle ≤60°. The linear array transducer was placed on the brachial artery proximal to the Hokanson pressure cuff. A 1-min baseline measurement of diameter and blood velocity was taken and the cuff was inflated to ≥ 250 mmHg for 5-min to fully occlude the artery. Full occlusion was confirmed when no pulse was found during palpation of the radial artery. After the 5-min occlusion, the cuff was released and followed by a 4-min recovery period to ensure optimal time for peak diameter in all participants. Brachial artery images were stored offline and diameters were analyzed using a commercially available edge-detection and wall-tracking software package (Vascular research tools 6, [Medical Imaging Applications, Coraville, Iowa]) as described previously (31). All baseline and post-cuff velocity (cm/s) values were averaged into 3 s bins using the manufacturers on screen software and time aligned with diameter to calculate FMD in both absolute (mmΔ) and relative (%Δ) values. In addition, shear rate was calculated as: [Shear rate (s^{-1}) = 4 x mean blood velocity (cm/s) / diameter (cm)]. The stimulus for arterial dilation was calculated as area under the shear rate curve (AUC_{SR}) using the trapezoid rule (72) and was used to normalize the FMD response.

Lower Body Negative Pressure (LBNP)

Participants were placed into a custom-built LBNP chamber in the supine position up to the iliac crest, as described previously (36). Once in the chamber, an initial test to was used to familiarize participants and confirm a proper seal. The level of LBNP (~30 mmHg) used in the current investigation has been confirmed to primarily unload the cardiopulmonary baroreceptors without altering arterial pressure and provides reproducible increases in muscle sympathetic nerve activity in the forearm (35). LBNP was used at rest (min 2 - 4) and during steady-state exercise (min 5 - 7) to observe vasoconstrictor responsiveness.

Sodium Intake and Nitrite Analysis

Participants were randomly assigned to consume either 15 g/day of sodium chloride (NaCl) or placebo (cellulose) for seven-days in the form of gel capsules. Participants were instructed to consume 1-2 pills every other hour to limit stomach upset. To confirm sodium intake, urine was collected prior to experiments on both days of testing. All urine was sent off for

a routine random sodium analysis (LabCorp, Burlington NC). Measurements of nitrite [NO_2^-] were performed within 30-min after samples had thawed via chemiluminescence with an Ionic/Sievers NO analyzer (NOA 280i, GE, Boulder, CO) in triplicate after instrument calibration, described in detail previously (73).

Experimental Protocol

All participants consumed either seven-days of HS or placebo (PL). After the first treatment condition participants went through a washout period and repeated the second treatment condition. Male participants went through a seven-day washout between conditions. Females went through a longer washout period due to hormonal changes and were tested during the early follicular phase of two consecutive menstrual cycles. All participants completed taking the pills up to the night of the seventh day, which was followed by laboratory testing the following morning. Importantly, all females began taking either supplement mid-way though menstruation, so they would be tested during the early-follicular phase as menstrual cycle may have an impact on sodium handling (74).

When participants first arrived in the laboratory, a urine sample was taken along with height and weight. Next, participants laid supine and the FMD protocol (see Experimental measurements) was performed. To obtain maximal voluntary contraction (MVC) participants had the right arm extended to the side at heart level (~ 80°) and were instructed to squeeze a handgrip dynamometer maximally for ~2 seconds. The MVC maneuver was repeated three times with ~2 min of rest between trials. The average of the two highest individual trials was used to obtain MVC and from this, 20% MVC was calculated. Next, the subjects got into the LBNP chamber and the seal was tested to confirm a steady negative pressure. Participants then completed a 4 min resting protocol: 2 min of baseline and 2 min of LBNP to assess resting vasoconstrictor responsiveness. After a 5 - 10 min recovery, participants underwent 7-min of hand-grip exercise at 20% MVC. During the final 2 min of exercise (min 5 - 7), the LBNP chamber was turned on to assess active vasoconstrictor responsiveness.

Data Analysis

Finapres derived blood pressures were calculated as minute averages at rest and during steady-state exercise. During the rest-to-exercise transition blood pressure was averaged across 10-second bins. Additionally, all forearm blood flow (FBF) and forearm vascular conductance (FVC) data were time aligned and averaged into 1-minute bins during resting and steady-state exercise measurements. In all cases, FBF was calculated as: FBF = mean blood velocity • 60 • π • (brachial diameter/2)^2 calculated in ml · min^{-1} (51). MAP was time aligned with FBF to calculate forearm vascular conductance (FVC) calculated in ml · min^{-1} · 100 mmHg [FVC = (FBF / MAP) * 100]. During resting conditions, FBF, FVC, and MAP measurements were averaged across the second minute (min 1-2) of rest and LBNP (min 3 – 4). During exercise, FBF, FVC, and MAP were calculated as steady-state values prior to LBNP (min 4 - 5) and during the final minute of exercise with LBNP (min 6 - 7). Functional sympatholysis was calculated as %ΔFVC = FVC– FVC_{ss})/ FVC_{ss} x 100, where ss denotes stead-state FVC (7, 39).

Statistics

Data were analyzed with commercially available statistical software package (Sigmaplot; version 12.5, Systat software, San Jose). SBP, DBP, MAP, FBF, FVC, ΔSV, $\Delta\dot{Q}$, ΔSVR were analyzed with a repeated measures ANOVA with a Bonferroni correction for pairwise comparison. Paired-sampled t-test was used for nitrite analysis. Functional sympatholysis was analyzed for between treatment significance with a t-test. The level of significance was set at ($p < 0.05$). All data were presented as means ± standard error.

Results

Hemodynamic responses at rest and during steady-state exercise

Urinary analysis revealed a significant increase in sodium excretion during the HS relative to control conditions (HS: 134±21; PL: 57±17 mmol/L; $p < 0.01$). However, no change was found between conditions with nitrite analysis (HS: 193±1.241; PL: 185±53 nmol L^{-1}; $p > 0.05$). Brachial artery FMD percent change was significantly reduced during the HS condition (HS: 8.5±1.2; PL: 13.1±1.4; $p < 0.01$). AUC_{SR} was not significantly different between treatments ($p > 0.05$). When the FMD was normalized to AUC_{SR} significant changes were still present (HS: $2.2±0.26^{e-5}$; PL: $4.1±0.56^{e-5}$; $p < 0.01$) (Figure 3.2).

Resting baseline and exercise steady-state SBP was significantly higher after the HS diet ($p < 0.05$). Similarly, steady-state MAP and DBP were significantly higher during HS ($p < 0.01$). The RPP was not significantly different at rest (HS: 7846±493; Pl: 7472±517 mmHg/beat; $p > 0.05$); however, the RPP was significantly greater during steady-state exercise (HS: 9588±686; Pl: 8749±649 mmHg/beat; $p < 0.05$). The rate-of-rise in DBP at 10, 50, and 60 seconds and throughout the first minute of exercise for MAP were significantly higher ($p < 0.05$) during the HS condition (Figure 4.2), but not SBP ($p > 0.05$). The change from baseline HR, SVR, stroke volume, and cardiac output were not different between conditions ($p < 0.05$) (Table 3.2).

The FBF and FVC responses during steady-state exercise were not different between conditions ($p > 0.05$) (Figure 5.2). Interestingly, LBNP caused a significant decline in FVC at rest during the placebo condition ($p < 0.05$), but not during HS ($p > 0.05$). During steady-state exercise functional sympatholysis was shown as the decrease in FVC evoked by LBNP was significantly different from baseline ($p < 0.05$). However, during exercise the degree of functional sympatholysis was not significantly different between conditions ($p > 0.05$; Figure 6.2).

Discussion

The purpose of the current investigation was to evaluate the effect of a HS load on the cardiovascular responses during exercise. These data clearly show that a HS diet (15 g/day) increased absolute blood pressure at rest and during exercise. Further, we show than the RPP was elevated with HS intake during steady-state exercise. However, HS intake did not appear to exaggerate the relative change in MAP (exercise pressor reflex) or impair functional sympatholysis when LBNP was initiated, noted by the similar percent change in FVC during exercise (Figure 6.2). These results provide insight into the exaggerated cardiovascular responses during HS intake and exercise.

Investigations targeting the blood pressure response during exercise have consistently shown that an exaggerated blood pressure response increases the risk of CVD (75-77). Partially in agreement with our first hypothesis, we show that 5-7 days of HS intake increased the absolute blood pressure response (Figure 4.2), but not the exercise pressor response (ΔMAP: HS 8mmHg; PL 5mmHg) during handgrip exercise. We also show that the RPP was significantly elevated during exercise in the HS condition, leading to increased work on the heart and risk of an adverse cardiac event (78). The current investigation is at odds with Yamauchi, Tsuchimochi (57) who have recently shown in Sprague-Dawley rats, that three-weeks of a HS diet (4.0% NaCl) increased the exercise pressor response during static hind-limb contractions. Further, Mizuno, Downey (59) have shown that aldosterone and HS intake independently augment both type III and IV afferent receptors of the exercise pressor reflex. It is unknown why our results differ from previous findings, but this finding may be linked to the length of HS intake being too short. Another question that remains unknown is which mechanism drives the increased pressor response with HS intake (57, 59). It is suggested that aldosterone may share a common pathway (59) or that a sensitization of the rostral ventrolateral medulla play a major role (57, 79, 80).

It is well known that endothelial-dependent vasodilation is attenuated in hypertensive patients and individuals consuming large amounts of dietary salt (56, 60, 62, 81). The current investigation showed an attenuated FMD response in patients undergoing HS intake (Figure 3.2). Further, because high salt may increase blood volume (82), we corrected for AUC_{SR} and still showed significant decreases to FMD. These results are in line with previous work showing salt

driven decrements to endothelial-dependent vasodilation (60, 61). This finding is clinically relevant in that endothelial dysfunction is thought to be the first step to development of atherosclerosis (83). The decrements to the FMD response may be multifactorial, but the FMD protocol followed (see experimental methodology) suggests that the endothelial-dependent vasodilation is primarily NO mediated (83, 84). While the current study did not measure changes in oxidative stress, changes in [NO_2^-] were not shown between conditions. This is interesting and it is not known why HS led to lower FMD in the face of unchanged [NO_2^-]. Previous work has shown that HS intake impairs vascular relaxation in rat aortas, and that tempol, a superoxide scavenger, lead to improvements in the percent relaxation to methacholine, an acetylcholine agonist (65). The impairment in FMD highlights the effect on large conduits with HS intake; however, further work is needed to observe changes in [NO_2^-] over time.

In addition to decreases in endothelial-dependent vasodilation increases in sympathetic nerve activity are known to occur with acute salt loading (79). Recent work suggests a role for hypothalamic (85) and rostral ventrolateral medulla (86) mechanism in mediating the acute salt loading increases in sympathetic nerve activity, with a potential role for increased baroreflex activity (87) carotid body activity (88). da Silva et al. (2018) demonstrated that removal of the hypothalamus abolished the acute salt load induced increases in sympathetic nerve activity, but was significantly attenuated with only carotid body removal, suggesting a role for both central and peripheral mechanism mediating this increase. This increased sympathetic nerve activity may be contributing to the increased absolute arterial blood pressure observed in the present study. Brian et al. (2018) demonstrated significant increase muscle sympathetic nerve activity during handgrip exercise following intravenous infusion of sodium chloride (79). This supports previous work in animal models demonstrating a direct correlation between the magnitude of the sympathetic nerve activity and arterial blood pressure responses with acute salt loading (85).

The current investigation utilized LBNP during steady-state exercise to observe the impact of HS intake on functional sympatholysis. In contrast to our hypothesis we did not observe any impairment in functional sympatholysis during steady-state exercise in the HS condition. Interestingly, resting vasoconstrictor responsiveness was reduced with HS intake (Figure 6.2). This result was unanticipated, but may be due to a compensatory, shear-stress

induced, increase in endothelial NOS (eNOS). Ying and Sanders (89) observed, after 48 hours of salt loading, an increase in rat aortic and renal cortex eNOS due to increases in vessel shear stress. In the current investigation baseline AUC_{SR} was not significantly higher in the HS condition (HS: 54±11; PL: 44±8; p=0.4). However, resting FBF was similar pre/post LBNP, suggesting that the percent change in FVC was driven by the salt-induced increase in MAP (Figure 4.2). In contrast to resting conditions, during steady-state exercise, the LBNP evoked percent change was not significantly different between conditions (Figure 6.2). This finding is noteworthy given previous work has identified oxidative stress as a mechanism leading to impairments in functional sympatholysis (67). This finding may suggest that the HS intake did not increase oxidative stress to a level that may impair functional sympatholysis or that the current treatment protocol was not long enough to disrupt this mechanism.

Limitations

In the current investigation we did not measure 24-hour ambulatory blood pressure and therefore could not classify subjects as salt-sensitive or salt-resistant. Additionally, we did not measure oxidative stress, renin, angiotensin, or aldosterone in the current study. This may have caused us to miss valuable information with regard to salt-sensitivity. Finally, the current investigation was not designed to suggest a specific sensory arm (i.e., type III/IV) of the exercise pressor reflex, central command or changes in baroreflex sensitivity.

Conclusion

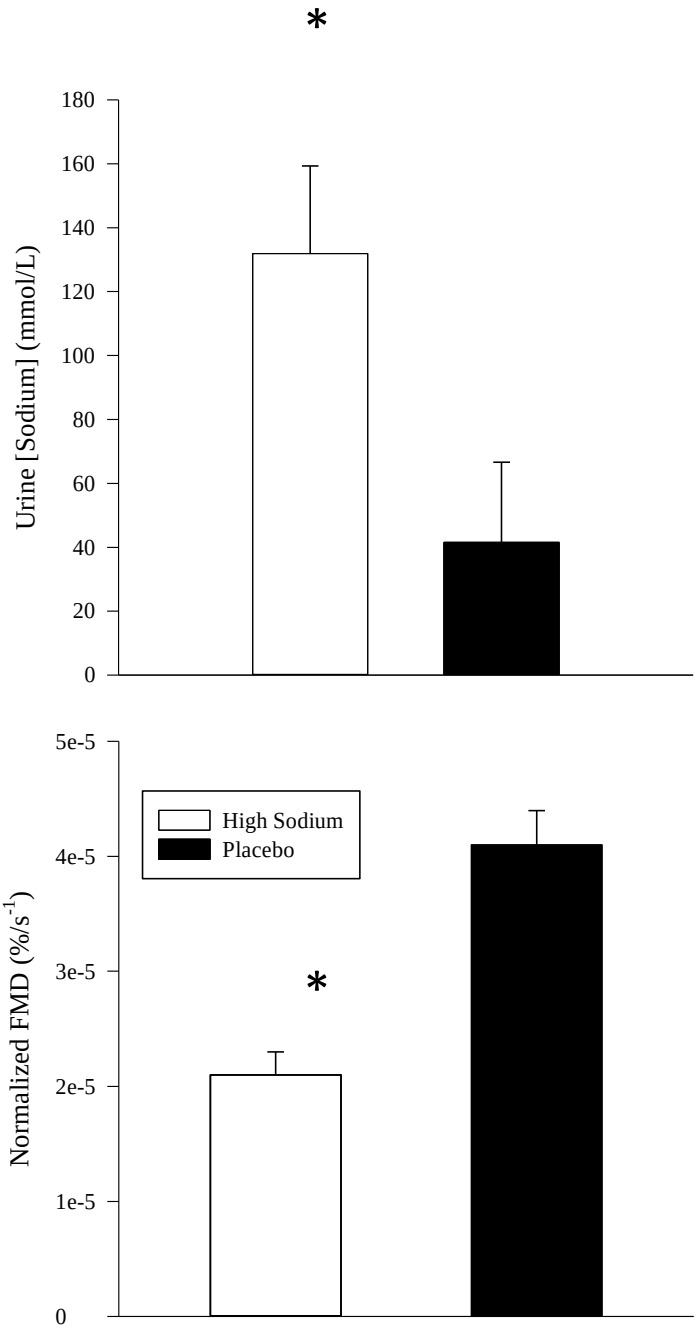
In conclusion, we provide the first evidence that 5-7 days of HS intake augments the absolute blood pressure response and RPP to rhythmic hand-grip exercise. We also show that 5-7 days of HS intake did not impair functional sympatholysis. While the specific mechanisms behind the response are currently unknown, these data highlight the impact of sodium intake and the detrimental cardiovascular outcomes during the rest-to-exercise and steady-state exercise conditions. Over time, exaggerated changes in MAP and RPP during exercise likely lead to increased incidence of hypertension, acute myocardial infarctions and CVD. Beyond baseline blood pressure measurements, investigations focused on salt intake must also consider the impact of dietary salt on cardiovascular responses during exercise. As such, these data are important given that salt intake is a key mediator of all-cause morbidity and mortality (90).

Table 3.2 Cardiovascular responses

	Placebo			High Sodium		
Baseline						
Heart rate (bpm)	59	±	2	57	±	3
Systemic vascular resistance (mmHg L min^{-1})	18.3	±	1.1	19.3	±	1.4
Stroke volume (ml min^{-1})	95	±	5	97	±	5
Cardiac output (L min^{-1})	5.5	±	1.3	5.4	±	1.1
Mean arterial pressure (mmHg)	96	±	4	102	±	4
20%						
Heart rate (bpm)	63	±	2*	62	±	3*
Systemic vascular resistance (mmHg L min^{-1})	18.4	±	1.2	19.8	±	1.6
Stroke volume (ml min^{-1})	93	±	5	96	±	5
Cardiac output (L min^{-1})	5.8	±	1.3	6.1	±	1.1
Mean arterial pressure (mmHg)	102	±	4	11	±	4
Δ rest-to 20%						
ΔHeart rate (bpm)	4	±	1	5	±	1
ΔSystemic vascular resistance (mmHg L min^{-1})	0.1	±	0.1	0.5	±	0.3
ΔStroke volume (ml min^{-1})	-2	±	2.8	-1	±	2.4
ΔCardiac output (L min^{-1})	0.3	±	0.28	0.7	±	0.97
ΔMean arterial pressure (mmHg)	5	±	1	8	±	1

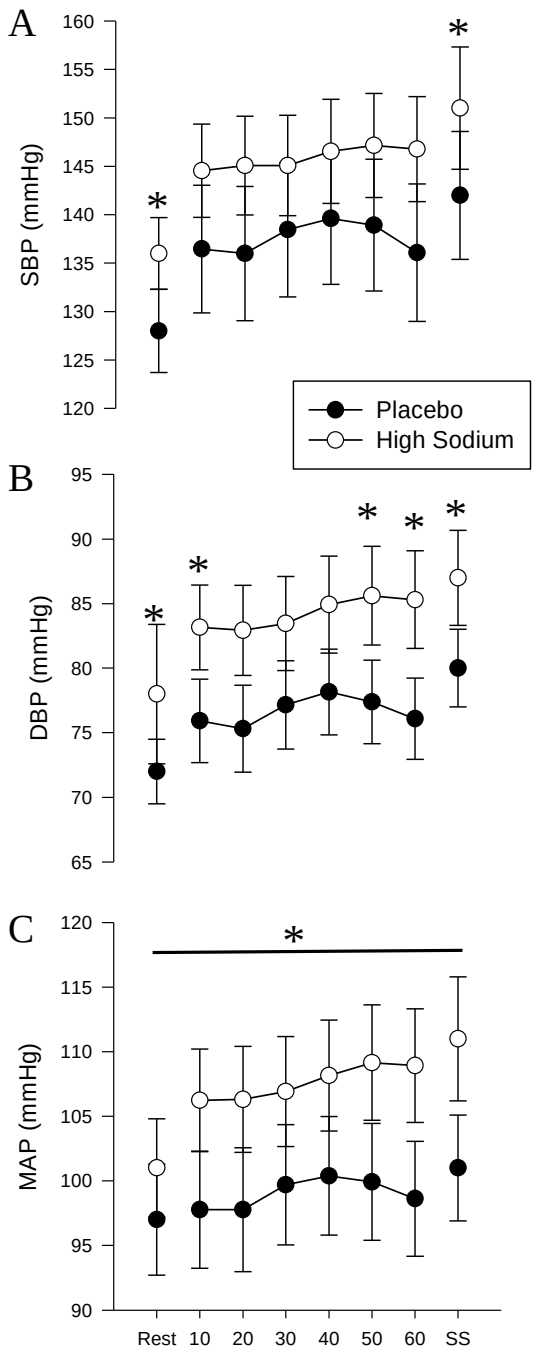
* Denotes increase from baseline ($p<0.05$)

Figure 3.2 Urine sodium and Flow Mediated Dilation



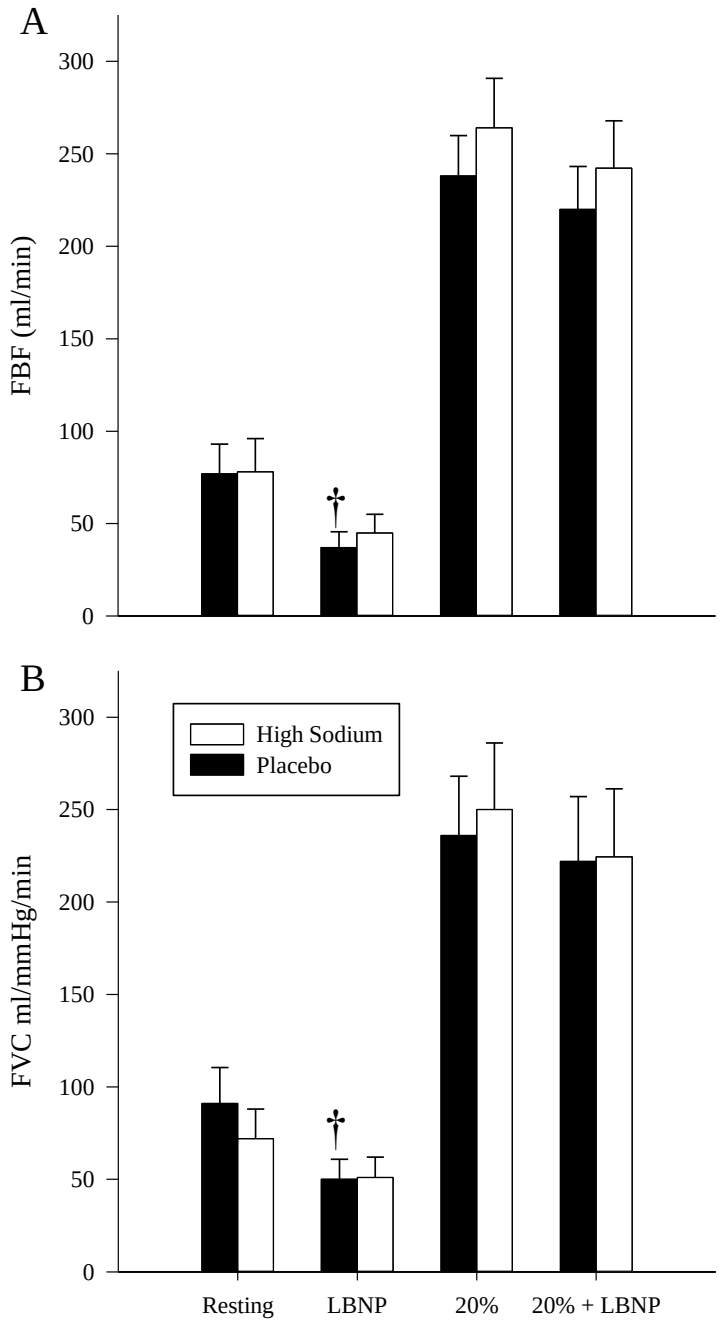
Pre/post (A) Urine sodium; and (B) Normalized endothelial-dependent flow-mediated dilation response. All data are means ± standard error. * denotes significant difference between conditions ($p<0.01$).

Figure 4.2 Finapres derived blood pressure



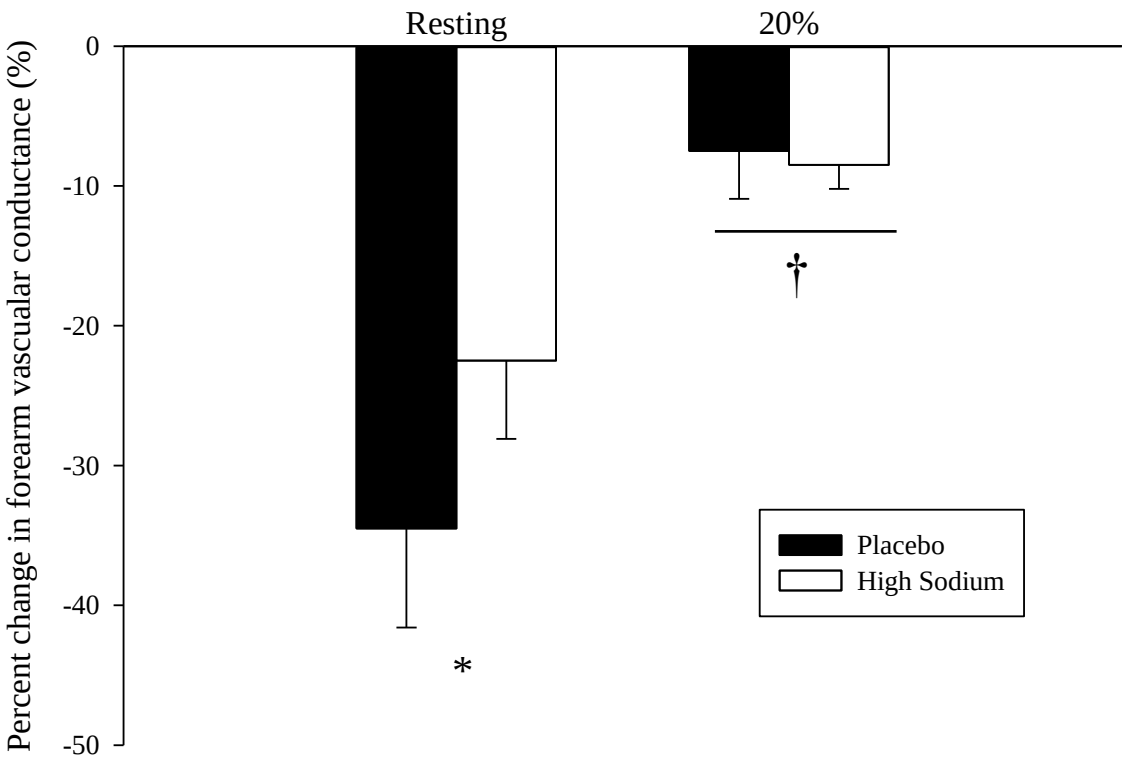
(A) systolic, (B) diastolic, and (C) mean arterial pressure at rest, during the first minute of exercise and steady-state exercise. * denotes significant difference between conditions ($p<0.05$).

Figure 5.2 Forearm blood flow and vascular conductance



(A) forearm blood flow; (B) forearm vascular conductance at rest, and during steady-state exercise. Both conditions include lower body negative pressure responses. All data are means ± standard error. † denotes significant difference due to LBNP ($p<0.05$).

Figure 6.2 Functional sympatholysis during high sodium and placebo



Percent change in forearm vascular conductance between high salt and placebo conditions. All data are means ± standard error. * denotes significant difference within conditions. † denotes significant difference from rest. No significant difference between conditions during 20% ($p>0.05$).

Chapter 4 - Impact of Acute Dietary Nitrate Supplementation During Exercise in Hypertensive Women

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Summary

The aim of the current investigation was to examine if dietary nitrate supplementation would improve vascular control in hypertensive post-menopausal women (PMW). We tested the hypotheses that acute dietary nitrate supplementation would 1) significantly decrease arterial blood pressure (BP) at rest and during exercise; 2) increase limb blood flow during steady-state exercise; and 3) improve functional sympatholysis during steady-state (SS) exercise. Ten hypertensive PMW underwent a randomized, double-blind placebo-controlled trial with a nitrate-rich (NR) or nitrate poor (NP) supplement. Beat-by-beat BP and heart rate were recorded throughout the trial on the non-exercising limb. Forearm blood flow (FBF) was measured via ultrasonography on the brachial artery of the exercising limb. All patients performed a resting CPT (2 min) and then 7 min of submaximal hand-grip exercise with a CPT applied during min 5-7. SS systolic (NR: 170±7; NP: 171±37 mmHg), diastolic (NR: 89±2; NP: 92±2 mmHg) and mean arterial (NR: 121±4; NP: 123±2 mmHg) pressures were not different between NP and NR treatment conditions ($p>0.05$). During SS exercise, FBF (NR:189±8; NP: 218±8 ml min^{-1}; $P=0.03$) in the NR treatment was significantly lower compared to NP. During min 6-7, the percent change in FVC was improved by ~50% with the NR supplement. In summary, an acute NR supplement improved functional sympatholysis by ~50% versus a NP placebo condition. Improvements in functional sympatholysis may have important implications regarding exercise tolerance in hypertensive PMW.

Introduction

Recent guidelines estimate that 46% of the U.S. adult population has hypertension (HTN) (91). A condition that places them at an increased risk of developing overt cardiovascular disease (CVD) (91). Regular exercise training is a common therapeutic strategy used in hypertensive patients to lower CVD risk by improving both aerobic fitness and vascular control (91, 92). However, individuals with HTN have an exaggerated increase in heart rate (HR) and blood pressure (BP) during acute bouts of exercise compared to their normotensive counterparts (93). This abnormal increase in HR and BP during exercise in hypertensive individuals can be hazardous as it increases the risk of stroke, acute myocardial infarction, or sudden cardiac death (94). Pharmacological treatment of HTN has consistently been shown to lower BP and subsequent incidence of CVD development (95). However, the use of non-pharmacological therapeutic strategies (e.g., dietary nitrate supplementation) alone or in combination with standard treatment practices such as increasing physical activity, lowering salt intake or weight loss are limited (96-98). This combination therapy with dietary nitrate may provide additional improvements in the BP responses during exercise which would aid in the prevention of an exercise-induced cardiovascular event (99).

It is now well understood that HTN elicits vascular dysfunction via decreased nitric oxide synthase (NOS) activity and subsequent decreases in nitric oxide (NO) bioavailability (13, 17, 19). As such, individuals with HTN are a potential target population for non-pharmacologic therapeutic interventions that increase NO bioavailability. Third-generation beta-blocker therapies have recently been shown to alleviate skeletal muscle ischemia in hypertensive patients during exercise (17), which may be due to increases in NO bioavailability (100). Similarly, Kapil, Khambata (97) showed that non-pharmacological supplementation with chronic dietary nitrate supplementation, which increases NO bioavailability, lowered 24-hour ambulatory BP in both treated and untreated patients with hypertension. Further, dietary nitrate acts independently of endothelial NOS (eNOS) activity leading to increases in forearm blood flow during hypoxic exercise in older normotensive individuals (101). These investigations highlight the beneficial impact of dietary nitrate supplementation and its ability to improve blood flow in both healthy and at-risk populations. Given these effects of acute dietary nitrate supplementation, the potential for a combination therapeutic strategy in patients with HTN seems promising.

HTN is linked to a marked decrease functional sympatholysis during sympathetically mediated vasoconstriction (35), likely due to a reduction in NO bioavailability (19). Functional sympatholysis is defined as a diminished vasoconstriction during increases in sympathetic activity within the active skeletal muscle (3). Pharmaceutical agents like angiotensin receptor blockers and beta-1 blockers have been shown to significantly improve the ability of the vasculature to overcome increases in sympathetic outflow to the active skeletal muscle in patients with HTN, improving skeletal muscle blood flow (17, 19, 102). However, beyond improvements with exercise training (103), non-pharmacological treatment options without notable side-effects have yet to be identified in patients with HTN to improve functional sympatholysis (19, 104). Specifically, it remains unknown if increasing NO bioavailability, via acute dietary nitrate supplementation, alters the balance of sympathetically mediated vasoconstriction in the contracting musculature of hypertensive postmenopausal women (PMW). This is critical given that the exaggerated vasoconstriction to increases in sympathetic nerve activity is now recognized as a key contributor to skeletal muscle malperfusion (10).

To date, no investigations delineating the impact of acute dietary nitrate supplementation during exercise in hypertensive PMW have been performed. Therefore, the purpose of the current investigation was to use a randomized, double-blind, placebo-controlled crossover study design to test the hypotheses that, compared to placebo, acute dietary nitrate supplementation would 1) significantly decrease arterial blood pressure at rest and during exercise; 2) increase forearm blood flow during steady-state exercise; 3) improve functional sympatholysis during steady-state (SS) exercise.

Methods

Participants

Ten PMW, [age 56 ± 3 years (mean ± SE); height 165 ± 2 cm; mass 84 ± 13 kg; body mass index 31 ± 5 kg/m^2], diagnosed with hypertension according to their primary care physician volunteered to participate in the current investigation. Women were classified as post-menopausal based on questions from the health history forms; all patients tested were at least one year from the last menstrual cycle. Additionally, patients were relatively sedentary with the majority of physical activity spent walking. Six of the ten patients were taking an angiotensin-II blocker + diuretic. Another two were on a beta blocker, and the other two patients were on either an angiotensin-II blocker + diuretic + Ca blocker or a beta blocker + angiotensin-II blocker. Importantly, new American Heart Association guidelines place these patients as stage two hypertensive patients (91). During the investigation, all patients were told to maintain their current diet, continue taking anti-hypertensive medication, and refrain from all alcohol consumption, mouthwash, and chewing gum 24 h before testing (105). Current patients were screened prior to data collection for diabetes and regular tobacco use and were excluded if having either. Written approval and confirmation of hypertension from primary care physicians coupled with verbal and written informed consent from each patient was obtained following approval from the institutional review board for research involving human subjects at Kansas State University; all work conformed to the *Declaration of Helsinki*.

Experimental Protocol

The current investigation was a two-day randomized, double-blind, placebo-controlled crossover study design. Patients were randomly assigned to either a nitrate-rich (NR) or nitrate-poor (NP) supplement in the form of beetroot juice. Two-hours prior to testing sessions, patients orally consumed either 140 ml of concentrated NR [NO_3^-] beetroot juice supplement (BEET IT Sport, James White Drinks Ltd, Ipswich, UK) containing [12.9 mmol NO_3^-], or 140 ml NP placebo beetroot juice supplement (PL; James White Drinks Ltd, Ipswich, UK) containing negligible [NO_3^-].

To obtain maximal voluntary contraction (MVC) patients had the right arm extended to the side at heart level (~ 80°) while lying supine and were instructed to squeeze a handgrip dynamometer maximally for ~2 seconds. The MVC maneuver was repeated three times with ~2 min of rest between trials. An average of the two highest individual trials was used to obtain the patient's MVC and calculate 20% MVC, used on both experimental days (table 4.3). Next, a 20-gauge venous catheter was placed in the left antecubital vein for measurement of plasma nitrite. Patients then rested quietly for at least 10 minutes followed by two cold pressor tests (CPT). The CPT was performed by an investigator passively moving the patients left leg into a bucket of ice-water (~2-3 degrees Celsius). During the CPT ice-water was continuously circulated to limit heat buildup around the foot and the water level was maintained around the distal portion of the shin. The first CPT (not shown in Figure 8.3) was used as familiarization and the second CPT served as the resting vasoconstrictor response (Figure 8.3). After baseline FBF and BP measurements, the patients began 7-min of dynamic hand-grip exercise on a custom-built hand-grip dynamometer in the right arm at a rate of 20 contractions min at 20% MVC. Steady-state measurements prior to the CPT were taken at min 4-5. Patients continued to exercise and the CPT was applied (min 5 – 7). During the CPT, water temperature was maintained at 2 – 3°C. All testing was completed in a temperature-controlled laboratory (20-25 °C) at the same time of day between treatment conditions.

Experimental Measurements

Venous blood samples were drawn at rest and placed into lithium heparin vacutainers (Becton Dickenson, NJ) centrifuged at 3300 RPM at room temperature for 20-min and immediately stored at -80°c for later analysis in determining circulating plasma nitrite levels ($[NO_2^-]$). Measurements of $[NO_2^-]$ were performed within 30-min after samples had thawed via chemiluminescence with an Ionic/Sievers NO analyzer (NOA 280i, GE, Boulder, CO) in triplicate after instrument calibration, described in detail previously (106).

Beat-by-beat HR, mean arterial (MAP), systolic (SBP) and diastolic blood pressure (DBP), were continuously measured via finger photoplethysmography (Finometer Pro, FMS, The Netherlands), calibrated to brachial artery blood pressure according to manufacturer specifications. Importantly, calculations were used within the validated model that utilizes a

Modelflow algorithm, as previously described, to measure changes in stroke volume (SV) and cardiac output ($\dot{Q}$) (69, 70). Systemic vascular resistance was calculated as MAP divided by $\dot{Q}$. Rate pressure product (RPP), and index of myocardial work, was calculated as HR ×SBP. Measurements of brachial artery diameter and blood velocity were simultaneously measured with an ultrasound system (LOGIQ S8, GE medical systems, Milwaukee, WI) equipped with a multi-frequency linear array transducer operating at 10 MHz, placed ~10 cm proximal from the antecubital fossa with care taken to avoid the bifurcation of the artery. All measurements had a Doppler sample volume set at the full width of the vessel with the insonation angle <60°. Brachial artery images were stored offline and diameters were analyzed using a commercially available edge-detection and wall-tracking software package (Vascular research tools 6, [Medical Imaging Applications, Coraville, Iowa]), described previously (107).

Forearm blood flow (FBF) was calculated as: FBF = mean blood velocity • 60 • π • (brachial diameter/2)^2 calculated in ml · min^{-1}. Mean arterial blood pressure (MAP) was time aligned with FBF to calculate forearm vascular conductance (FVC) calculated in ml · min^{-1} · 100 mmHg [FVC = (FBF / MAP) * 100] (29, 108). All Finapres derived variables were averaged across the second minute of rest before CPT and during the second minute of CPT. Additionally, MAP, SBP, and DBP were averaged across the final minute of steady-state exercise (min 4 – 5), and during the final minute of exercise + CPT (min 6 – 7). Functional sympatholysis at rest and during exercise was calculated as the percent reduction in FVC from the steady-state (ss) values 30 s before starting the CPT and during the nadir of each CPT: %Δ FVC = $FVC_{nadir} – FVC_{ss})/ FVC_{ss}$ x 100 (Figure 8.3). This method is suggested as the ideal method for analysis of the vasoconstrictor response (7, 39).

Statistics

Data were analyzed with a commercially available statistical software package (Sigmaplot; version 12.5, Systat Software, San Jose). Plasma [NO_2^-] values were compared between conditions as a paired-samples t-test. SBP, DBP, MAP, FBF, FVC, ΔSV, $\Delta\dot{Q}$ were analyzed with a two-way repeated measures ANOVA with Student-Newman-Keuls post hoc for pairwise comparison. The level of significance was set at ($p < 0.05$). All data are presented as mean values ± standard error.

Results

Plasma blood sample

Plasma nitrite levels were significantly higher during the nitrate-rich treatment (809 ± 146 nM) than the placebo (79 ± 19 nM) condition (Figure 9.3; $p < 0.001$).

Blood pressure response

Resting SBP, DBP, HR, MAP, and SVR were not significantly different between treatments ($p > 0.05$) (Table 5.3). Resting MAP was significantly increased during the CPT in both NR and NP treatments ($p < 0.01$) but no differences were found between treatments ($p = 0.67$). When the CPT was applied during steady-state handgrip exercise, MAP ($p < 0.01$) significantly increased above steady-state MAP but was not different between NR and NP treatments ($p = 0.67$). The rate of rise (i.e., first minute in the rest-to-exercise transition) in HR, SBP, DBP, SVR, and RPP were not significantly different between NR or NP treatments ($p > 0.05$). The absolute change in the MAP, SBP, DBP, and RPP from rest to exercise were not different between NR and NP treatments ($p > 0.05$).

Systemic hemodynamics

FBF at rest was not significantly different between treatments (NR: 63 ± 10, NP: 62 ± 11 ml min^{-1}; $p > 0.05$). FBF during steady-state exercise was reduced 13% following the NR compared to the NP treatment (NR: 190 ± 16, NP: 218 ± 17 ml min^{-1}; $p = 0.03$; Figure 10.3). FVC at rest was not significantly different between treatments (NR: 59 ± 8, NP: 58 ± 7 ml mmHg min^{-1}; $p > 0.05$). FVC during steady-state exercise was reduced 10% following the NR compared to the NP treatment (NR: 159 ± 12, NP: 177 ± 12 ml mmHg min^{-1}; $p = 0.03$ Figure 10.3). The use of a CPT at rest elicited a significant reduction in both FVC and FBF ($p < 0.01$), shown in figure 10.3. The CPT significantly lowered steady-state FBF during the NP treatment by ~11%; ($p = 0.03$) but did not significantly lower FBF in the NR treatment ~2.0%; ($p > 0.05$) (Figure 10.3 A). The CPT significantly reduced steady-state FVC in the NP treatment (~15%; $p = 0.01$); and in the NR treatment (~7%; $p = 0.01$) (Figure 10.3 B). During steady-state exercise,

the percent decrease in FVC evoked by the CPT was attenuated in both treatments (Figure 11.3 A), however the NR treatment provided an additional ~50% increase compared to the NP treatment (Figure 11.3 B).

Discussion

The principle findings of the current investigation suggest that acute dietary nitrate supplementation does not lower BP or increase the FBF response at rest or during steady-state exercise in hypertensive PMW. Interestingly, FBF and FVC during the final minute of steady-state exercise were significantly lower in the NR compared to the NP treatment conditions. Further, a key finding to the current study was shown by an improvement in functional sympatholysis by ~50% with a NR supplement (Figure 11.3 B). The improvement in functional sympatholysis with acute nitrate supplementation may provide a novel method of improving vascular control during exercise in hypertensive PMW (109, 110).

Blood pressure response to hand-grip exercise

During exercise, a healthy normotensive individual will maintain, or slightly increase, perfusion pressure (e.g., MAP) across the arterial system. The finding of a significant pressor response (~18 mmHg) to hand-grip exercise is nearly-double that of previous work in older individuals performing hand-grip exercise at similar intensities (101). This highlights the impact of HTN and the associated increase in the risk of having an exercise-induced cardiovascular event (91). The current investigation found that MAP, SVR, SBP, DBP, HR, and RPP were increased from baseline during steady-state hand-grip exercise (Table 5.3). However, these responses were not different between NR or NP conditions. Importantly, Trinity, Layec (111) found that post-menopausal women have a significantly exaggerated SVR response to single leg knee extension exercise; however, in younger women no increase in SVR was observed. The current finding of an increase in SVR during exercise in the face of an unchanged $\dot{Q}$ is interesting and supports the findings of Trinity, Layec (111). It has been suggested that the vasculature in younger women is “protected” from increases in muscle sympathetic nerve activity (MSNA) as the hormonal effects of estrogen augment NOS activity and this activity aids in attenuating the vasoconstriction (33). However, post-menopausal women lack estrogen, giving MSNA a larger role in regulating SVR (112). Thus, strategies aimed at increasing NO bioavailability, like chronic dietary nitrate supplementation (97), may be efficacious in restoring normal vascular

control during exercise. However, in the present study, an acute NR supplement did not attenuate the increase in SVR during steady-state exercise, which likely contributed to the observed BP responses (Table 5.3).

The findings of the present study are somewhat at odds with previous work investigating the therapeutic effects of nitrate supplementation. For example, lower peak exercise SVR reserve in heart failure patients has been shown previously with acute dietary nitrate supplementation (113). However, it is currently unknown why there was not a significantly lower BP at rest and during steady-state exercise with a NR treatment. This finding is likely multi-factorial and may be due to the anticipation of the exercise test, the acute (2 min) resting BP measurement compared to the gold-standard 24-hour ambulatory BP measurement, or acute versus chronic nitrate supplementation (97). The clinical implications are two-fold. First, the potential therapeutic benefit of dietary nitrate may not be achieved with a single concentrated dose before exercise. Further, when our results are viewed in combination with previous work (97), it is suggested that chronic supplementation may be required to achieve clinically significant benefits in hypertensive PMW (98). Second, the potential added benefit of combination therapy with acute dietary nitrate is not supported by our current findings. This further supports the need for future investigations utilizing chronic versus acute nitrate supplementation in the management of hypertension, particularly during exercise.

Blood flow response to hand-grip exercise

The ability to redirect blood flow to contracting skeletal muscle during steady-state exercise is an important mechanism to allow prolonged physical exertion (2). Older women, however, have an attenuated ability to increase skeletal muscle perfusion in the face of increased sympathetic neural outflow (114, 115). Non-pharmacological interventions aimed at increasing blood flow to the contracting skeletal muscle in older populations, particularly those with hypertension would be beneficial (17). Dietary nitrate supplementation has been shown increase exercise tolerance during treadmill testing in patients with PAD (116), and increase blood flow in healthy aged humans during hypoxic exercise (101). In the present investigation, an acute NR supplement did not increase forearm blood flow during steady-state exercise when compared to the NP treatment condition. This finding is notable given the aforementioned reports in heart

failure and PAD patients supporting the use of a NR supplement (113, 116). In particular, we provide the first evidence that a NR supplement lowered the FBF and FVC during hand-grip exercise in hypertensive PMW at the same absolute exercise workload (Figure 10.3). The finding of lower steady-state FBF and FVC without subsequent differences in steady-state $\dot{Q}$ or SVR is the first of its kind and it may be linked to improved muscle contraction efficiency with dietary nitrate supplementation (117). More work is needed as previous investigations in young healthy animals and humans have shown that a NR supplement increases hindlimb (118), and forearm (119) blood flow, respectively, while the latter study also showed an increase in VO_2 to accompany the increase in blood flow. Finally, the current investigation did not measure oxygen consumption or provide any kinetic evaluation during hand-grip exercise so further comparison is limited.

Functional sympatholysis during a cold pressor test

Current evidence suggests a significant role for NO as a key moderator of sympathetically mediated vasoconstriction in both health and disease (6, 17, 19, 68, 108, 120, 121), but this is not without controversy (20, 21). We provide the first evidence that dietary nitrate supplementation increases functional sympatholysis in hypertensive PMW. These improvements support existing investigations that suggest NO bioavailability is an essential step in mediating sympathetic vasoconstriction during exercise (6, 17-19). For example, Price, Raheja (17) studied hypertensive patients that underwent chronic treatment with either Nebivolol or Metoprolol. They showed that Nebivolol, but not Metoprolol, improved functional sympatholysis, suggesting that NO, and not antioxidants, play a significant role in functional sympatholysis (17). The current study extends these findings by suggesting that improvements in NO bioavailability, via dietary nitrate supplementation, improves functional sympatholysis. In addition, because most pharmaceutical treatments have side effects (96, 122), and that there are no known side effects or drug interactions with dietary nitrate, the use of dietary nitrate in combination with medication may be a viable option during exercise.

Interestingly, certain patients in the current investigation with a larger percentage decrease to FVC during the NP treatment tended to have larger improvements with the NR treatment (Figure 11.3 B). It may be due to the increased efficacy of nitrate being converted

under lower O_2 tension; this may help mitigate ischemia prone tissue as shown in healthy individuals (123) and rats (124). The nitrate-nitrite-nitric oxide (NO_3^- - NO_2^- - NO) pathway produces NO via the reduction of inorganic nitrate (i.e., in the form of beetroot juice) to NO, and works independently from endogenous pathways, (i.e., endothelial nitric oxide synthase activity (eNOS) (104, 123)). The independence of this pathway is important when endogenous NO production is reduced as is the case in cardiovascular disease and HTN, leading to decreases in NO bioavailability via increased scavenging of NO by reactive oxygen species (13, 62, 93, 104, 125). This investigation supports the use of dietary nitrate as a non-pharmacological intervention in this population to enhance functional sympatholysis.

In a recent report, it was shown that metabolic rate, and not contractile 'work,' was a key determinant behind functional sympatholysis (28). In the current investigation, we have demonstrated that acute dietary nitrate supplementation lowered FBF and FVC (Figure 10.3) during steady-state handgrip exercise. The lower steady-state FBF response in the NR treatment is unlikely the reason for altered functional sympatholysis seen in this investigation. For example, Kruse et al. (2017) have demonstrated that decreasing handgrip contraction frequency (i.e., 10 vs. 20 contractions/min) at a matched workload led to a lower FBF response and less functional sympatholysis. Thus, we would have expected the lower steady-state FBF response in the NR treatment to result in greater vasoconstriction during the CPT. However, we observed improvements in functional sympatholysis during NR treatment when the CPT was applied suggesting that the increased NO bioavailability helped to attenuate the vasoconstriction. Further, it may be that the lower blood flow led to a greater buildup of metabolites, leading to the improvements in functional sympatholysis shown. However, metabolites were not measured in the current study and caution is needed until future work confirms the FBF responses.

Experimental considerations

Several important points must be considered when interpreting the findings of the present study. First, resting baseline BP data was collected in the supine position for two minutes prior to hand-grip exercise and should not to be directly compared with previous reports utilizing 24-hour ambulatory BP measurement. Second, the current investigation utilized hand-grip exercise, not lower limb exercise, which may not translate to ambulatory activities (e.g., walking). There

are also reports of different BP responses during upper or lower limb exercise (126). However we show increased BP and SVR during hand-grip exercise, and this in line with a previous report utilizing single leg knee extension exercise [25]. The current investigation did not use an older normotensive control group to compare responses and can only indirectly confirm an abnormal BP response to exercise. In addition, the current investigation used an acute dose of dietary nitrate supplementation. Future work should include chronic nitrate supplementation to investigate its therapeutic potential in altering arterial blood pressure during steady-state exercise. Finally, we did not measure sympathetic nerve activity in the current investigation. Recently, it has been shown that dietary nitrate may lower muscle sympathetic nerve activity during static handgrip exercise (110). As such, the use of nitrate supplementation could have had variable effects on sympathetic activity during dynamic handgrip exercise and during the CPT in our current population and must be considered with the current findings.

Conclusion

In summary, acute dietary nitrate supplementation did not reduce the BP response to hand-grip exercise in hypertensive PMW. Interestingly, FBF and FVC during steady-state hand-grip exercise at 20% MVC were significantly lower in the NR compared to the NP treatment condition. Additionally, it is suggested that increasing NO bioavailability may enhance functional sympatholysis and maintain blood flow during rhythmic handgrip exercise. The ~50% improvement in functional sympatholysis with a NR supplement during exercise may help increase exercise tolerance in hypertensive PMW. These findings suggest that the potential therapeutic benefit of dietary nitrate may be limited when a single concentrated dose prior to exercise is used in PMW. Further investigations will be required to determine if acute dietary nitrate supplementation can be used to improve other aspects of vascular control during exercise in those with HTN.

Table 4.3 Subject Characteristics

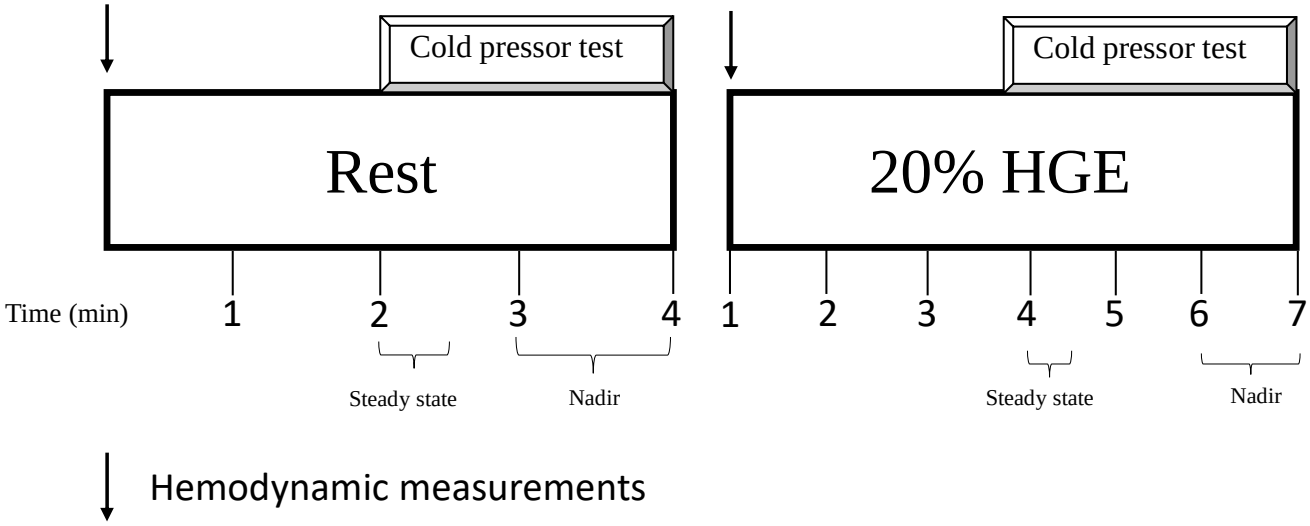
Subjects (n)	10		
Age (years)	56	∓	3
Height (cm)	165	∓	2
Weight (kg)	84	∓	13
Maximal Voluntary Contraction (kg)	35	∓	3

Table 5.3 Systemic hemodynamic measurements

	Nitrate-rich			Nitrate-poor		
BASELINE						
Heart rate (bpm)	64	±	1	63	±	2
Systolic blood pressure (mmHg)	145	±	5	145	±	3
Diastolic blood pressure (mmHg)	79	±	4	80	±	3
Mean arterial blood pressure (mmHg)	105	±	4	105	±	2
Rate pressure product (HR x SBP)	9289	±	387	9181	±	345
Systemic vascular resistance (mmHg L min^{-1})	18.81	±	1.08	18.72	±	1.59
Stroke volume (ml min^{-1})	91	±	5	93	±	10
Cardiac output (L min^{-1})	5.8	±	0.32	5.7	±	0.28
20% EXERCISE STEADY-STATE						
Heart rate (bpm)	68	±	2 #	69	±	2 #
Systolic blood pressure (mmHg)	170	±	7 #	171	±	3 #
Diastolic blood pressure (mmHg)	89	±	2 #	92	±	2 #
Mean arterial blood pressure (mmHg)	121	±	4 #	123	±	2 #
Rate pressure product (HR x SBP)	11562	±	702 #	11728	±	376 #
Systemic vascular resistance (mmHg L min^{-1})	22.02	±	1.34 #	21.66	±	1.12 #
Stroke volume (ml min^{-1})	87	±	6	85	±	5
Cardiac output (L min^{-1})	5.8	±	0.42	5.7	±	0.27

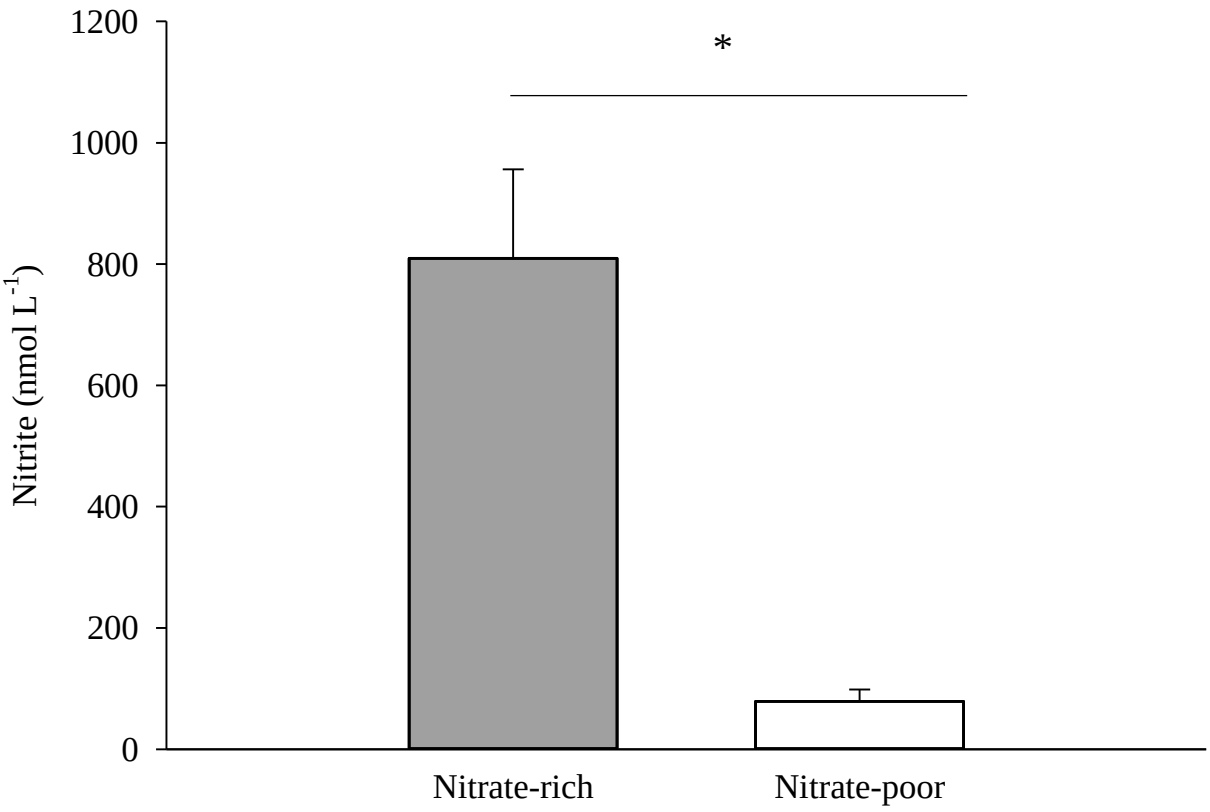
# denotes significant difference from baseline in same condition ($p < 0.05$). All data are means ± SE

Figure 7.3 Experimental design



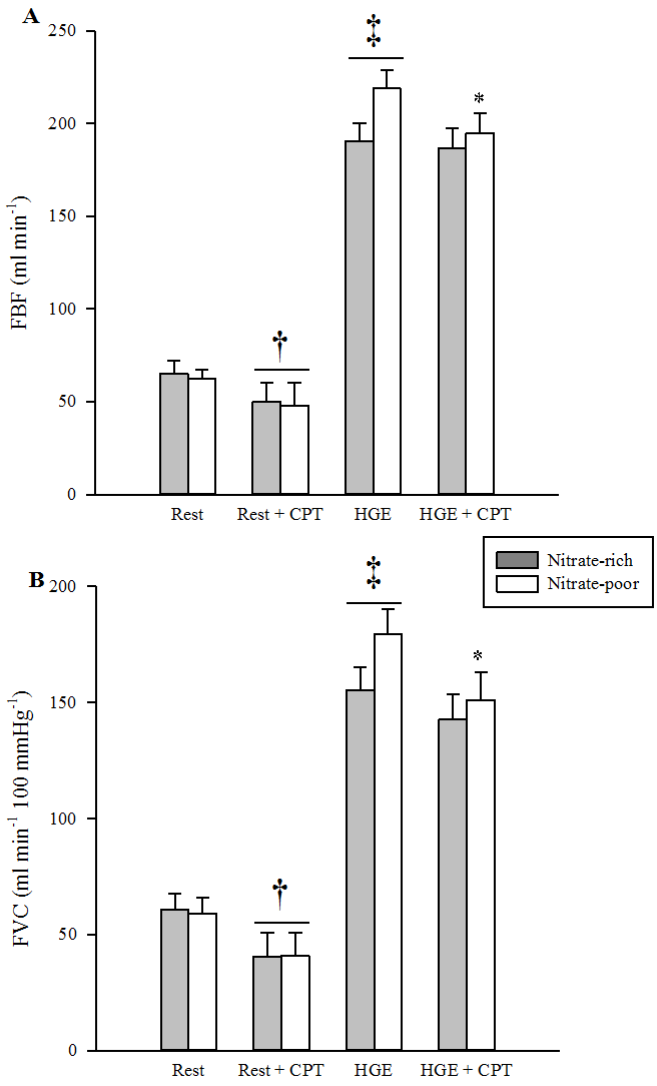
Timeline representing rest with and without cold pressor test; and exercise with and without the cold pressor test. Hemodynamic measurements were taken throughout the protocol. Steady-state and nadir measurements are used in the analysis (see methods).

Figure 8.3 Plasma nitrite analysis in each treatment



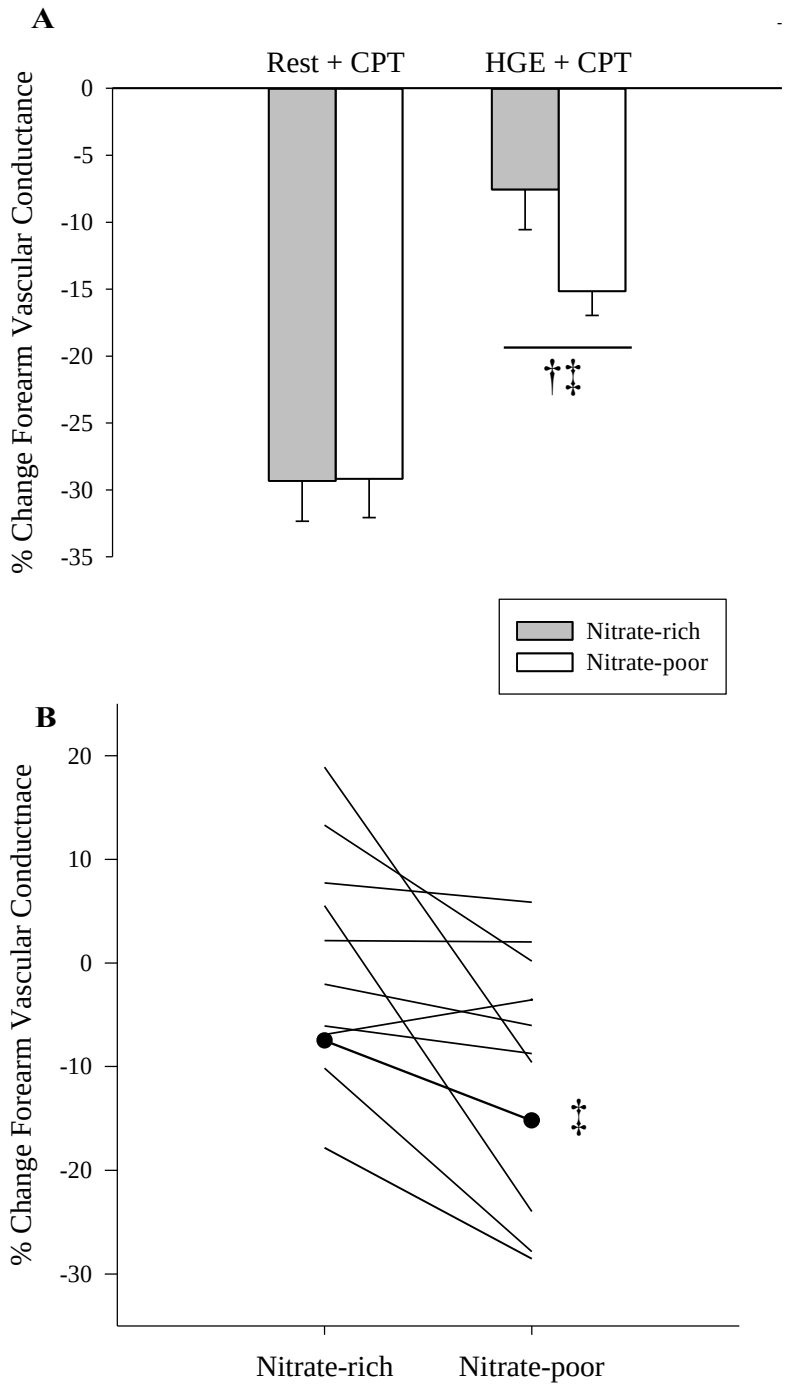
Data are means ± standard error * denotes significant difference between treatment conditions ($p < 0.01$).

Figure 9.3 Mean steady-state forearm blood flow and vascular conductance



(A): steady-state forearm blood flow and (B): forearm vascular conductance at rest and during hand grip exercise at 20% MVC. † denotes significant reduction due to cold pressor test ($p = 0.01$). ‡ denotes significant difference between treatment conditions ($p = 0.03$). * denotes significant reduction due to CPT ($p < 0.05$). Data are means ± standard error.

Figure 10.3 Forearm vasoconstriction during cold pressor test



A: Percent change in forearm vascular conductance at rest and during exercise in both treatments. **B:** Individual plots showing the percent change in FVC during exercise in both treatments. † denotes a significant attenuation in % change FVC from rest. ‡ denotes significant difference relative to nitrate-poor condition. Data are means ± SE.

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126. Boushel R. Muscle metaboreflex control of the circulation during exercise. Acta Physiol (Oxf). 2010;199(4):367-83.

CURRICULUM VITAE

NAME: Caldwell, Jacob T.

POSITION TITLE: Doctoral Candidate

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Eastern Michigan University	BS	2011	Physical Education
Eastern Michigan University	MS	2014	Exercise Physiology
Kansas State University	Ph.D.	Dec 2018	Kinesiology

Scientific Objective

My current work is dedicated to improving health and vascular function in post-menopausal women with hypertension and discovering if dietary nitrate supplementation can improve vascular responsiveness during exercise. My primary interests deal with the control of blood pressure and blood flow, endothelial function, and interventions to improve endothelial cell function with dietary nitrate supplementation in both health and disease.

Academic Appointments

2016 – 2018	Graduate Teaching Assistant in Department of Kinesiology, Kansas State University, Manhattan, KS
2014 – 2016	Graduate Teaching Assistant in Department of Health and Ex. Science, University of Oklahoma, Norman, OK
2011 – 2014	Graduate Teaching Assistant in Department of Exercise Physiology, Eastern Michigan University, MI

National Societies and Memberships

2017 –	American Heart Association
2016 –	American Physiological Society
2012 –	American College of Sports Medicine
2012 – 2014	National Strength and Conditioning Association

Teaching Experience

Instructional Experience: Kansas State University

Exercise Physiology Laboratory: KIN 336

Undergraduate (n = 10) course consisting of various physiology labs (e.g., metaboreflex, oxygen uptake kinetics, $\dot{V}O_{2max}$, autonomic control)

Measurement and Research Techniques Laboratory: KIN 310

Undergraduate (n = 20) course consisting of research methodology and techniques to enhance competence in collection of data and ability to find relevant literature

Behavioral Basis of Physical Activity Laboratory: KIN 220

Undergraduate (n = 20) overview of exercise physiology, biomechanics, and behavioral sciences

Grants Awarded

2017 – Kansas State University Doctoral Dissertation Research Grant ($1,000.00)

2016 – The University of Oklahoma: Robberson Travel Grant ($1,000.00)

Grants (not funded)

2018 – American College of Sports Medicine Pre-doctoral Award ($5,000)

2017 – American Heart Association Pre-doctoral Training Award ($52,000)

Journals Reviewed

2017 – Reviewer, Respiratory Physiology and Neurobiology

2018 – Reviewer, Journal of Human Hypertension

Leadership, Committees, & Awards

2018 –	Kansas State University – American Kinesiology Association, Doctoral Scholar Award
2017 –	Kansas State University – President, Kinesiology Association of Graduate Students
2014 – 2015	The University of Oklahoma, Graduate Student Council

Peer-Reviewed Publications

4. Ederer AK, Didier KD, Reiter LK, Brown, MG, Hardy R, Caldwell JT, Black CD, Larson RD, Ade CJ. Influence of adjuvant therapy on endothelial and microvascular function in the years following cancer treatment. PLOS One. 2016 Jan; doi:10.1371
5. Caldwell JT, Wardlow GC, Branch PA, Macarena R, Black CD, Ade CJ. Effect of exercise-induced muscle damage on vascular function and skeletal muscle microvascular deoxygenation. Physiol Rep. 2016, Nov;4(22)
6. Didier KD, Reiter LK, Ederer AK, Brown, MG, Hardy R, Caldwell JT, Black CD, Bemben M, Bemben D, Ade CJ. Peripheral vascular responses to small muscle mass exercise in cancer survivors treated with adjuvant therapy. Journal of the American Heart Association. 2017, Feb 7;6(2)
7. Smith JR, Sutterfield SL, Baumfalk DR, Didier KD, Hammer SM, Caldwell JT, Ade CJ. Left Ventricular Strain Rate is Reduced during Voluntary Apnea in Healthy Humans. J Appl Physiol. 2017 Sep 1
8. Hammer S, Didier K, Alexander A, Sutterfield SL, Caldwell JT, Ade CJ, Barstow TJ. (2017) Perfusive and diffusive oxygen transport in skeletal muscle during incremental handgrip exercise JAPPL-00815-2017.
9. Ramos Gonzalez, M., Caldwell, J.T., Branch, P.A., Wardlow, G.C., Black, C.D., J Campbell, R Larson, Ade, C.J. Impact of shear rate pattern on post-occlusive near-infrared spectroscopy microvascular reactivity Microvascular Research (In Press).
10. Frye, J. N., Sutterfield, S. L., Caldwell, J. T., Behnke, B. J., Copp, S. W., Banister, H. R., & Ade, C. J. (2018). Vascular and autonomic changes in adult cancer patients receiving anticancer chemotherapy. *J Appl Physiol (1985), 125*(1), 198-204. doi:10.1152/japplphysiol.00005.2018
11. Caldwell JT, Sutterfield SL, Craig JC, Baumfauk DR, Copp CW, Ade CJ. Impact of Acute Dietary Nitrate Supplementation during Exercise in Hypertensive Women (In review; MSSE)
12. Caldwell JT, Sutterfield SL, Post HK, Lovoy GM, Hammer SM, Alexander AM, Barstow TJ, Ade CJ. Vasoconstrictor Responsiveness Through Alterations in Relaxation Time and Metabolic Rate during Rhythmic Handgrip Contractions (In review; Physiological Reports)
13. Sutterfield, S. L., Caldwell, J. T., Post, H. K., Lovoy, G. M., Banister, H. R., & Ade, C. J. (2018). Lower cutaneous microvascular reactivity in adult cancer patients receiving chemotherapy. *J Appl Physiol (1985), 125*(4), 1141-1149. doi:10.1152/japplphysiol.00394.2018
14. Caldwell, JT, Post, HK, Lovoy, GM, Sutterfield, SL, Banister, HR, Ade, CJ. Impact of High Sodium Intake on Blood Pressure and Functional Sympatholysis during Handgrip Exercise (In review; Hypertension)

Scientific Presentations

1. Primary components of running kinematics differ with body mass index. 2012 MIACSM Annual Meeting. Gaylord, MI

2. Improved economy and acceleration profiles among high school runners following eight weeks of plyometric training. ACSM's 61st Annual Meeting World Congress on Exercise is Medicine, and World Congress on the Role of Inflammation in Exercise®. Orlando, Florida May 27-31, 2014
3. Practical guidelines of near-infrared spectroscopy at rest and during exercise. Experimental Biology 2018, San Diego.

Conference Abstracts: **Total = 30**