THE INFLUENCE OF CHRONIC AND ACUTE SODIUM LOADING ON BLOOD PRESSURE REGULATION IN HEALTHY NORMOTENSIVE ADULTS

by

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A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Applied Physiology

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LIST OF ABBREVIATIONS

24h, 24-hours

ARV, average real variability

AU, arbitrary units

AV3V, anteroventral third ventricle

BP, blood pressure

BMI, body mass index

BP, blood pressure

CO_2, carbon dioxide

DBP, diastolic blood pressure

F, female

HG, handgrip

HS, high sodium

IRB, Institutional Review Board

LS, low sodium

M, male

MAP, mean arterial pressure

MIN, minute

MnPO, median pre-optic nuclues

MSNA, muscle sympathetic nerve activity

MVC, maximum voluntary contraction

Na^+, sodium

NaCl, sodium chloride

O_2, oxygen

OVLT, organum vasculosum lamina terminalis

OsM, osmolality

PEI, post exercise ischemia

PVH, paraventricular nucleus of hypothalamus

RPE, ratings of perceived exertion

RS, run-in sodium

RVLM, rostral ventrolateral medulla

SBP, systolic blood pressure

SD, standard deviation

SFO, subfornical organ

TC, time control

TPR, total peripheral resistance

Yr, years

ABSTRACT

Excess dietary sodium intake increases cardiovascular disease risk in healthy normotensive adults independent of resting blood pressure (BP). Data using animal models suggest that high sodium (HS) in the diet alters BP regulation through serum sodium (SNa^+) sensing mechanisms in the brain, leading to increased BP variability and exaggerated exercise pressor reflex activation. Acute increases in SNa^+ in humans have been shown to increase resting BP and sympathetic nerve activity. No studies have addressed the time course (chronic vs. acute) effect of elevated SNa^+ on BP variability and BP responses during exercise in humans. **PURPOSE:** To determine whether chronic and/or acute increases in SNa^+ lead to greater BP variability and exaggerated BP responses during exercise pressor reflex activation. **HYPOTHESIS:** Chronic increases in SNa^+ (i.e., 7 days), but not acute increases in SNa^+ (23 minute hypertonic saline infusion), would increase BP variability and augment BP responses during exercise pressor reflex activation. **METHODS:** Chronic high SNa^+ was accomplished by having normotensive participants complete 7 days of low sodium (LS; 20 mmol Na^+/day) and 7 days of HS (300 mmol Na^+/day) in random order, and 24h BP variability and BP responses to exercise pressor reflex activation (40% handgrip exercise followed by post exercise ischemia) were assessed on the last day of each diet. Acute high SNa^+ was accomplished using a 23 minute infusion of 3%

hypertonic saline; a time control (TC) trial was also performed. Four and a half minutes of BP variability was assessed prior to and following the acute infusion, as was BP and muscle sympathetic nerve responses to exercise pressor reflex activation. **RESULTS:** Chronic increases in sodium consumption increased 24h BP variability in only those that had an increase in SNa^+ >2 mmol/L (n=24; LS: 10.9±0.5, HS= 12.9±0.6 mmHg, $p<0.05$). Acute increases in SNa^+ had no effect on BP variability (n=18; 4.4±0.5 vs. 4.3±0.4 mmHg, $p>0.05$). In response to exercise pressor reflex activation, chronic increases in SNa^+ increased systolic BP responses during exercise pressor reflex activation (n=16; LS=137.2±4.1 mmHg vs. HS=145.9±4.1 mmHg, $p<0.05$). While acute increases in SNa^+ raised sympathetic outflow (pre=11±2 vs. post=15±2 bursts/min, $p<0.05$) but had no effect on BP responses to exercise pressor reflex activation ($p>0.05$). **CONCLUSION:** Chronic increases in SNa^+ increase 24h BP variability and systolic BP responses to exercise pressor reflex response activation, while acute increases in SNa^+ have no effect on short-term (4.5 minutes) BP variability or BP responses during exercise pressor reflex activation. These data provide evidence that alterations in SNa^+ influence BP regulation in normotensive adults.

Chapter 1

REVIEW OF LITERATURE

1.1 Introduction

Dietary sodium has been studied closely over the past forty years. Individuals that have increased blood pressure (BP) following consumption of high dietary sodium have been termed as salt-sensitive and have higher risks of cardiovascular disease and early mortality (105, 106). Roughly 51% of hypertensives and 26% of normotensives are thought to be salt-sensitive. This information has provided rationale for dietary guidelines to promote consuming lower dietary sodium (<2,300 mg). Despite changes to national dietary guidelines to promote lower averaged BP, many normotensive and hypertensive individuals still consume dietary sodium in excess. The estimated average daily intake of sodium is ~3,400mg/day (43). A large meta-analysis highlighted that high sodium (HS) consumers are at greater risk of hypertension, stroke and trended towards increased risk of cardiovascular events (94).

A meta-analysis performed by Strazzullo et al (94) provided evidence that progressively increasing sodium intake correlated with increased cardiovascular and stroke events in both hypertensive and normotensive populations. Recent studies in normotensive rats and humans have shown excess dietary sodium impairs vascular function (23, 35, 53), and more recent rat studies have shown high dietary sodium

increases cardiovascular reactivity to sympathetic and parasympathetic stimuli (1, 2, 86, 114). Both impaired vascular function and increased cardiovascular reactivity are attributed to increase cardiovascular and cerebral vascular disease risk (32, 98). However, studies in normotensive humans have not examined whether dietary sodium alters circadian BP regulation and BP responses to exercise. Therefore, the purposes of the following literature review were (1) to examine various measures of circadian BP patterns and the influence of chronic dietary sodium and acute increases in SNa^+ on circadian BP patterns, and (2) examine cardiovascular control of BP during exercise and the influence of chronic and acute increases in sodium on cardiovascular control during excess sodium intake. The following literature review is important because impaired circadian BP patterns, exaggerated cardiovascular responses, and excess dietary sodium have all been linked to future cardiovascular disease risk in normotensive humans. Currently, limited data in humans exist on whether chronic or acute increases in SNa^+ alter circadian BP patterns and BP responses to exercise.

1.2 Clinical Relevance Assessing Ambulatory BP Monitoring

The utilization of ambulatory BP monitoring has become a reliable instrument for capturing fluctuations in circadian BP patterns throughout daytime and nocturnal periods (65). Normal 24h BP follows a circadian pattern, rising to the highest point during the daytime periods and declining during sleep periods, followed by a upward surge upon waking (Figure 1.1). Several novel methods have been developed to

characterize these fluctuations BP over a 24h period. An early study by Mancia et al (55) measured intra-arterial BP recordings in hypertensive and normotensive patients to assess spontaneous changes in circadian BP patterns. The study found that in addition to hypertension, patients with high BP had increased BP variability (55). Since these early studies, BP variability has been demonstrated to be a predictor of future cardiovascular disease (39, 69, 80, 83), target organ damage (57, 84, 96), and mortality (61, 77).

Several methods have been used to calculate how variable BP is throughout a given time period (24h, daytime, nighttime). The most commonly used method calculates the standard deviation (SD) of mean BP (systolic, diastolic, and mean arterial pressure), which has been associated with target organ damage, future cardiovascular disease, and mortality (68, 69, 76, 77, 96). The SD method reflects the dispersion of BP away from the mean, but is associated with mean pressure. In light of this association with mean pressure, more recent papers have developed newer formulas (Average Real Variability index & Weighted Average Real Variability) to measure variability non-invasively through ambulatory BP monitors. Essentially, these newer methods average the absolute differences of consecutive BP measurements over a given time period removing the influence of mean BP and time between BP readings (39, 61).

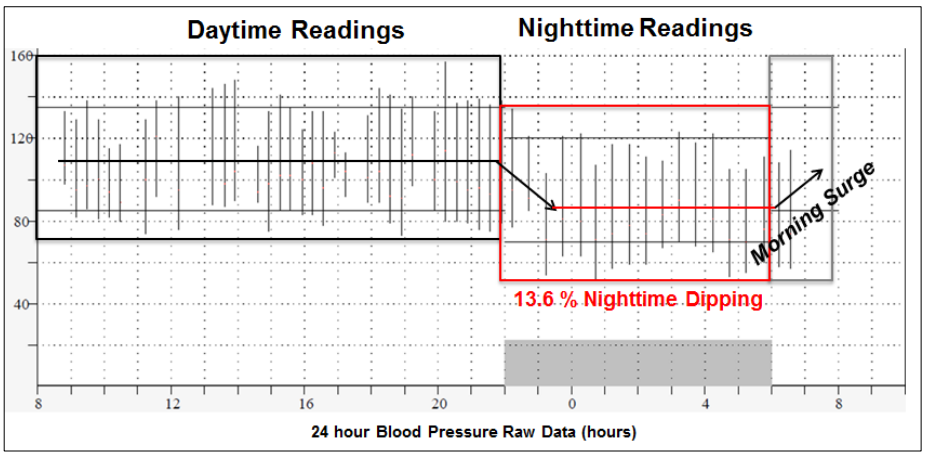

Figure 1.1: **24h ambulatory BP recording.** Circadian BP patterns observe daytime BP readings followed by a pronounced decrease in nocturnal BP (~10%) in healthy populations and then a morning surge in BP. The following figure is an original ambulatory BP recording.

Both indices (SD & ARV) of BP variability correlate with increased cardiovascular disease risk and mortality independent of mean BP in normotensive and hypertensive populations (38, 49, 57, 70, 96). Early observations utilizing the SD method found that for a given mean BP in individuals with untreated primary hypertension, those with greater fluctuations during half hour periods had greater target organ damage (70). Similarly in borderline hypertension, individuals with the highest daytime BP variability increased kidney damage and left ventricular hypertrophy despite no significant difference in mean daytime pressure (96). More recent studies have utilized ARV, which accounts for the mean difference between

subsequent BP measurements, which has been shown to predict cardiovascular outcomes in untreated and treated hypertensive populations (61, 75).

1.3 Dietary Sodium's Impact on Circadian BP Patterns in Normotensives

In animal studies, dietary sodium has been shown to augment circadian BP patterns (increased daytime BP variability) and augment BP responses to sympathetic stimuli (sciatic afferent stimulation, exercise pressor reflex, direction injections of sympathetic agonist into the rostral ventral lateral medulla) (1-3, 44, 71, 86). The measurement of BP variability reflects cardiovascular responses between environmental, behavioral, peripheral reflexes (baroreflex & exercise pressor reflex), daily activity, humoral, and circadian rhythmic influences (nocturnal decline in BP and pre-waking morning BP surge) (56). The following section reviews the impact of dietary sodium on circadian BP patterns (nocturnal dipping, daytime BP variability & morning surge) in non-hypertensive populations.

Ruddy et al (80) examined correlations between 24h urinary sodium excretion and 24h ambulatory BP measures. They observed several positive correlations between urinary sodium excretion and indices of systolic BP variability (standard deviation: daytime and nighttime; coefficient of variability: daytime and nocturnal) and diastolic BP variability (standard deviation: asleep; coefficient of variation: awake and sleep) (80). Further, studies have found pressure natriuresis influences 24h BP and nighttime mean BP (90). These studies highlight the relation between sodium excretion and BP, perhaps reflecting sodium handling in the kidneys. Previous studies

have demonstrated individuals with hypertension, salt-sensitivity, and who are of African descent are more likely to have impaired 24h circadian BP patterns (8, 100, 110). For instance, nocturnal BP typically declines 10-20% relative to mean daytime BP; however, impaired renal handling of sodium has been shown to increase nocturnal BP due pressure natriuresis occurring during nocturnal periods instead of daytime periods leading to impaired nocturnal dipping and elevated 24h BP (8). Despite studies suggesting dietary sodium impacts nocturnal sodium excretion and BP, other studies have found no impact of dietary sodium on nocturnal dipping in normotensives. Simonetti et al (87) detected no difference in nocturnal dipping in white healthy normotensive young adults, children, and in salt-sensitive subjects under high sodium conditions (87). This leads to the possibility that kidney dysfunction and race play a role in 24h BP during elevated levels of sodium intake.

In conclusion, these studies mentioned above highlight important implications for examining circadian BP patterns under varying sodium diets. Impaired circadian BP patterns (increased BP variability and blunted nocturnal dipping) have been associated with increased target organ damage and cardiovascular mortality (49, 67, 70). Importantly, research studies and review articles on circadian BP measures have offered potential limitations regarding small sample sizes and associations between BP variability and mean BP. Furthermore, there are still considerable inconsistencies establishing prognostic significance of BP variability (39, 56, 61, 65). Future studies are necessary to examine circadian BP patterns under well-controlled sodium diets, and should utilize established method guidelines to examine these patterns (39, 56).

High dietary sodium is recognized to have negative cardiovascular effects and increased mortality; and current research has not utilized a randomized trial to examine circadian BP patterns in normotensives under HS and low sodium diets (8, 80, 87).

1.4 Blood Pressure Control During Exercise and the Potential Role of Dietary Sodium

Cardiovascular responses during exercise work to redistribute blood flow away from inactive tissues to active tissues (88). Three neural control mechanisms contribute to the cardiovascular responses to exercise which supports blood flow to active tissues to provide nutrients (O_2, glucose, free fatty acids, etc.) and remove waste products (lactate, CO_2) (108). The three neural mechanisms responsible for altering cardiovascular responses throughout exercise are central command, the exercise pressor reflex, and the baroreflex (108). The following section reviews current literature pertaining to these three neural pathways involved during exercise. The review also examines the potential adverse exercise BP response during increased sodium loading in rats and humans.

1.4.1 Central Command

Central command provides feedforward control of cardiovascular responses stimulated by signals from the motor cortex or subcortical nuclei (33). These signals

are responsible for recruiting muscle motor units and activating parallel cardiovascular control centers in the brainstem, which control sympathetic (increases during exercise) and parasympathetic (withdraws during exercise) outflow during exercise (78, 88). Upon the start of exercise, BP and heart rate will increase relative to exercise intensity and continue to increase until active muscle blood flow needs are met or until fatigue during dynamic and static exercise (78, 79, 88). The relative contribution of central command on exercise appears to rely on exercise intensity. Victor et al (102) utilized neuromuscular blockades to inhibit muscular force generation and therefore required augmented central command activation of motor units in order to increase muscular force. The increased intensity (near maximal intensity) during the study produced robust increases in efferent sympathetic nerve traffic and large increases in heart, highlighting the role of central command to decrease parasympathetic activity controlling heartrate (102).

1.4.2 The Exercise Pressor Reflex

The exercise pressor reflex is a peripheral reflex mechanism, which provides sensory feedback to the brain about the mechanical and metabolic environment in muscle. Composed of group III (muscle mechanoreflex) and IV (muscle metaboreflex) afferent nerve fibers, the reflexes are sensitive to changes in mechanical distortion of muscle tissue and chemical environment alterations, respectively. During exercise, the activation of group III afferent fibers from stretch and pressure results in increased BP

and sympathetic nerve activity (40, 91, 109). Group IV afferent nerve fibers respond to changes in local muscle metabolite (lactic acid, H+, K+, bradykinin, arachadonic acid, and adenosine) concentrations produced during exercise, which results in an increased BP and sympathetic nerve response (48). Importantly, the activation of the exercise pressor reflex occurs independent of central command, which was elegantly demonstrated in decerabrated animal models. Upon activation, afferent nerve fibers transmit nerve signals back to the brain stem (88), terminating near the rostral ventral lateral medulla and nucleus tractus solitarii. Following afferent termination, the signal is integrated and efferent response is produced to cause corresponding cardiovascular responses (74).

1.4.3 Baroreflex

The afferent arm of the baroreflex is made up of stretch receptors (baroreceptors) located in the carotid sinus and aortic arch that respond to beat-to-beat changes in stretch. For instance at rest, increased aortic or carotid sinus stretch causes the activation of the baroreceptors and subsequent inhibition of sympathetic nerve activity lowering both BP and heart rate. Alternatively, as BP declines decreasing baroreceptor stretch, sympathetic outflow increases heart rate and increases peripheral resistance to raise BP (108). Therefore, the baroreflex provides short-term control of BP on a beat-to-beat basis. During exercise, the baroreceptors remain active, resetting to defend BPs at a higher level despite gradual increases in sympathetic nerve activity as exercise intensity increases (108). The resetting of the baroreceptors allows for

beat-to-beat alterations in heart rate and SNA, however the operating point that the baroreceptors typically operates at shifts upwards and the baroreceptors become less sensitive to changes in beat-to-beat pressure (Figure 1.2).

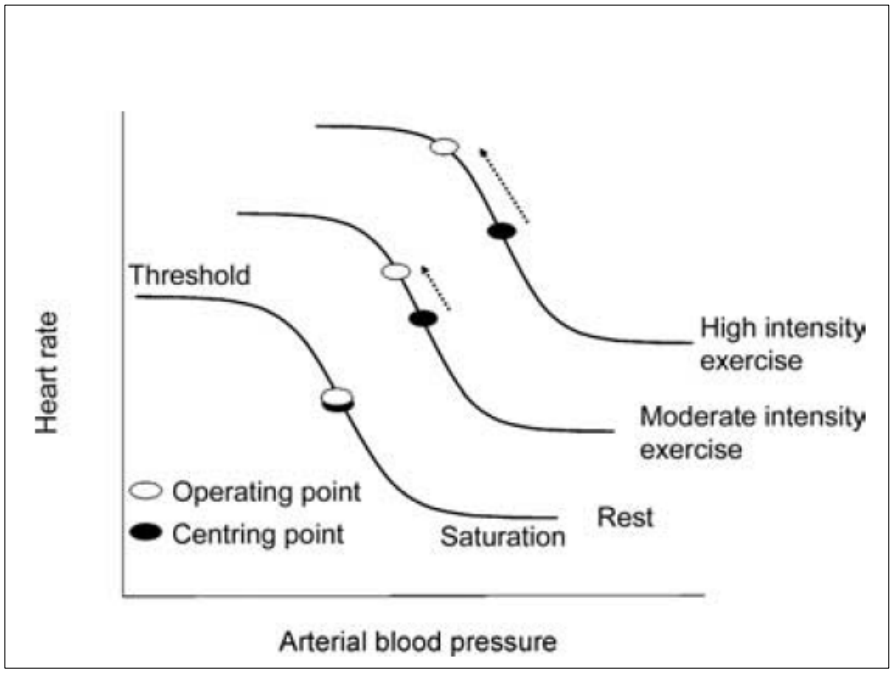

Figure 1.2 **Baroreflex Function at Rest and Exercise**. The following figure from Williamson et al (108) demonstrates intact baroreceptor resetting during exercise along sigmoidal curves. As exercise intensity progressively increases, the operating point shifts upward along the sigmoidal curve towards threshold of stimulus, allowing the baroreflex to further limit increases in pressure to sympathetic stimuli.

1.5 Dietary Sodium's Impact on Cardiovascular Control during Exercise in Normotensives

Studies in normotensive Sprague-Dawley rats have shown high dietary sodium alters central cardiovascular control centers within the brain (1, 2). The anteroventral third ventricle (AV3V) within the brain is exposed to several circulating factors due to an incomplete blood brain barrier, providing a potential site for sensing changes in circulating hormones, osmolality, and sodium (10). Water deprivation in rats has been shown to significantly increase plasma osmolality (water deprived rats: 308.2±0.7 vs. water replete rats 298.3±0.8, $p<0.05$), which was also found to elevate sympathetic nerve activity and mean arterial pressure. Following water deprivation, lowering osmolality via intracarotid infusion of hypotonic saline decreased sympathetic nerve activity and BP (14). In water replete rats, infusion of hypotonic saline had no effect on lumbar SNA and mean arterial pressure ($p<0.05$) remaining similar to baseline (14). These studies highlight the role sensing regions within the brain that sense high osmotic/sodium concentrations and altered sympathetic outflow controlling blood pressure regulation during acute increases in osmolality and sodium.

Previous studies in water deprived rats have provided insight into sodium sensing mechanisms within the AV3V region of the brain during acute high SNa^{+}. Studies utilizing forebrain lesions in rats have provided further empirical evidence into dietary sodium's impact on sodium sensing regions (1-3, 86). Lesions applied to AV3V regions of the brain removed structures believed to be involved in sodium sensing. Lesions applied to Sprague Dawley rats consuming both high sodium and low

sodium had normalized responses to sympathetic and parasympathetic stimuli compared to Sprague Dawley rats consuming high sodium (1). The rats consuming a HS diet, with no electrical lesions applied, had robust cardiovascular responses to sympathetic and parasympathetic stimuli. The exaggerated BP responses under HS suggests sodium sensing regions contribute to the robust cardiovascular responses by sensitization the rostral ventral later medulla (RVLM) as demonstrated in several studies (92). The sensitization of the RVLM is caused through slowly-developing alterations in neural plasticity in rats consuming dietary sodium longer than 14 days (92). Importantly, the RVLM is the primary integration site for feedforward and feedback control of various cardiovascular reflexes and BP during exercise. For instance, rats consuming excess sodium had exaggerated responses to sciatic afferent stimulation, aortic depressor nerve stimulation, acute volume expansion, and vagal afferent stimulation (86). Further, a recent study by Yamauchi et al (114) found that rats consuming excess sodium had augmented exercise pressor reflex responses during hindlimb stimulation. These studies in rats provide further evidence that excess sodium might exaggerate cardiovascular responses during exercise through sodium sensing mechanisms in humans (Figure 1.3).

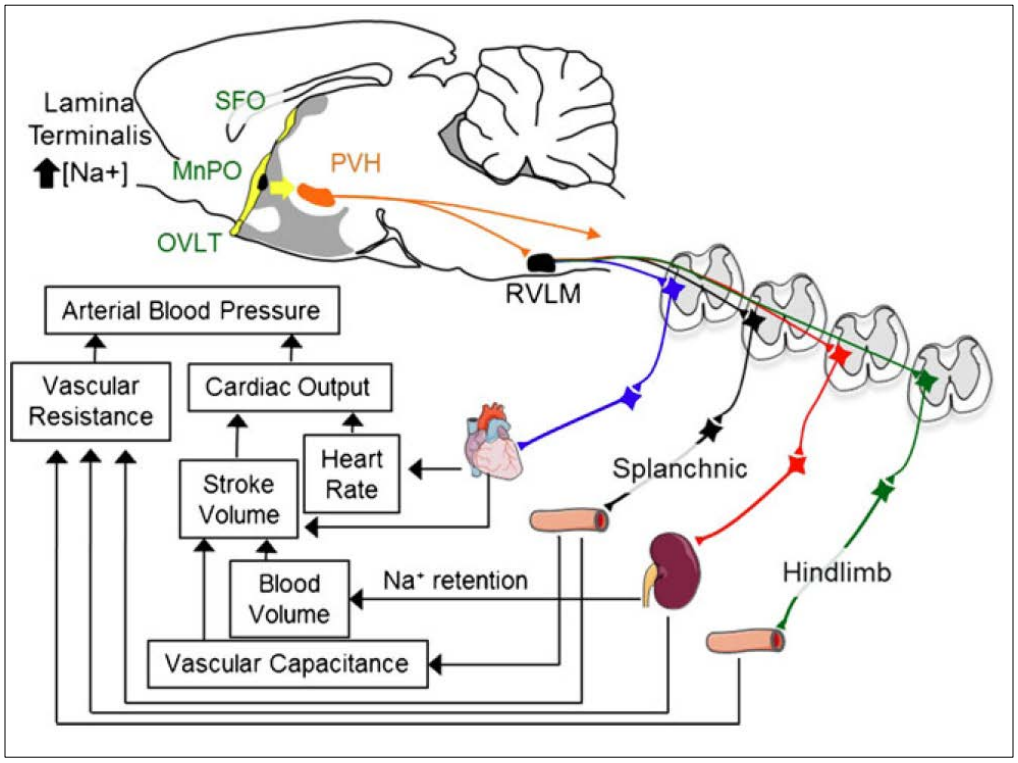

Figure 1.3 **Sodium's Interaction with the AV3V Region.** The following figure from Stocker, SD, et al (93), depicts sodium sensing regions of the organum vasculosum lamina terminalis, median pre-optic nucleus, and subfornical organ sense increases in sodium and osmolality. These neurons from the AV3V region influence sympathetic activity and blood pressure through polysynaptic projections to sympathetic preganglionic neurons, which influence vascular resistance, blood pressure, cardiac output, and blood volume.

In healthy normotensive adults, resting muscle sympathetic nerve activity is blunted during a HS diet compared to a very low sodium (LS) diet (6). During exercise, borderline hypertensive subjects consuming high dietary sodium were observed to have exaggerated BP responses to both cycling and handgrip exercise

despite no change in baseline BP between LS and HS diets (5). Taken together with findings in animal models isolating the exercise pressor reflex, it is reasonable to speculate that in humans, prolong HS intake potentially leads to exaggerated cardiovascular responses. In normotensives, exaggerated cardiovascular responses have been associated with increased risk of future cardiovascular events and development of hypertension (27, 59). Notably, no studies in humans have examined the necessary duration of sodium intake humans to develop enhanced cardiovascular responses during exercise.

1.6 Excess Sodium Exposure in Humans: Does length of exposure matter?

Excess sodium consumption is considered a major contributing factor to the pathogenesis of hypertension and has been associated with cardiovascular disease, morbidity, and mortality (81, 94, 104, 105). Animal studies have previously demonstrated that HS increases the risk of sodium induced hypertension, kidney dysfunction, vascular dysfunction, exaggerated cardiovascular responses, and target organ damage (brain, kidneys & heart) (45, 50-52, 92). The following section reviews the necessary length of exposure to HS that has been studied in animal and human studies.

Human studies have employed several study designs varying in duration to examine the implications of high dietary sodium on cardiovascular risks and prevention of cardiovascular disease. In order to effectively study mechanisms related to vascular function or cardiovascular responses to excess sodium or increases in

SNa^+, studies have measured these responses through acute saline infusions (36), water restriction (15), one HS meal (20, 21), and long term controlled feeding studies (≥7 days of HS) (6, 23, 35, 58). Studies acutely infusing hypertonic saline, found that increasing SNa^+ (Range: 1.9-2.8 mEq/L) and plasma osmolality (Range: 4-8 mOsm/kg H_2O) lead to increases in mean arterial pressure, increased sympathetic baroreflex sensitivity and increase resting muscle sympathetic nerve activity (26, 36, 107). In response to acutely increasing SNa, Greaney et al (36) observed no change to BP and muscle sympathetic nerve activity responses to hypoxia stimuli following hypertonic saline infusions, but did observe increased resting sympathetic nerve activity. Animal studies acutely increasing SNa^+ observe robust increases in sympathetic activity and pressor responses (11-14). Acute increases in serum sodium and osmolality were shown to increase resting muscle sympathetic nerve activity and modestly increase resting BP in normotensive adults (26, 36, 107). Alternatively to studies infusing NaCl to raise SNa^+, studies utilizing a HS meal found that excess sodium decrease vascular function (20). These studies provide evidence that acute increases in SNa^+ might ultimately lead to more chronic effects of HS and cardiovascular risk in normotensive adults. Acute sodium loading, similar to one high sodium meal (one high sodium meal = 65 mmol/L), observed significant reductions in flow mediated dilation of the brachial artery and increased augmentation index by radial applanation tonometry in healthy normotensives (20, 21). The acute sodium loading resulted in a 1.5 mEq/L increase in SNa^+, which is believed to cause significant reductions in nitric oxide bioavailability and nitric oxide synthase (20, 64).

Similarly, studies employing a chronic (≥ 7 day) HS diet observed significant reductions vascular function (conduit artery function & cutaneous microvascular function) compared to a 7 day LS diet in normotensive salt-resistant adults (23, 35, 53)and salt-sensitives adults (58). Longer durations (8 months to 5 years) of sodium restriction resulted in significant improvements in arterial stiffness in healthy normotensives compared to normotensive adults consuming HS diets (130 mmol/day-200 mmol/day). Regular intake of HS is related to increased BP variability and impaired nocturnal dipping, which has been associated with greater target organ damage (8, 80). Chronic increases in sodium load have been shown to adversely affect vascular function more so in normotensive salt-sensitive adults than salt-resistant adults. For instance, a DASH diet with HS was found to increase oxidative stress (urinary F2-isoprostane) & augmentation index in salt-sensitives and a DASH LS diet decreased oxidative stress and augmentation index; whereas salt-resistant normotensives had no change in measure of oxidative stress and augmentation index (4). Vascular function also appears to decrease more in men than compared to aged match females, opening the door to potential sex differences under acute and chronic conditions (53). These studies highlight that prolonged sodium exposure impairs vascular function, increases BP variability and exaggerated cardiovascular responses during exercise. These studies also provide insight into the chronic effect of HS on sex and normotensive sub populations (salt-resistant vs salt-sensitive).

Both chronic and acute increases in SNa+ appear to impact the cardiovascular system. The impact on chronic dietary sodium appears to alter vascular function

significantly, while limited data exists on the effects of elevated SNa^+ on the autonomic nervous system in normotensive humans. Unpublished data from our lab group has observed that SNa^+ can increase significantly from consumption of HS for 7 days (Figure 1.4). Future studies are needed to expand research focusing the effects of prolonged elevated SNa^+, as demonstrated in animal studies, on BP regulation in normotensive and hypertensive populations because of the increased cardiovascular disease risk associated with high dietary sodium.

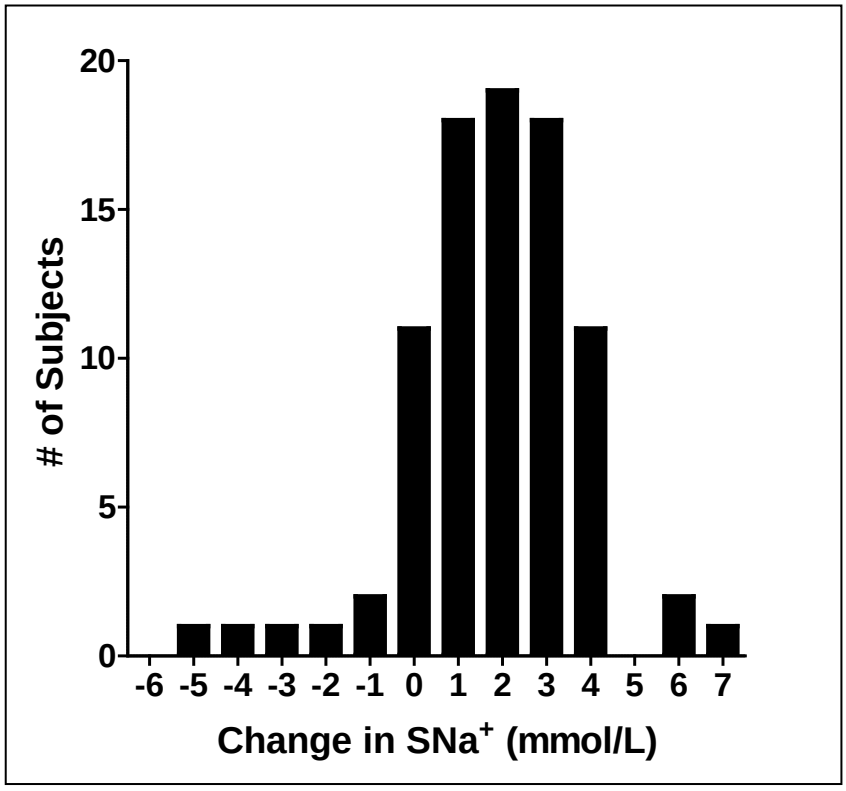

Figure 1.4 **Histogram showing the change in SNa^+ from a low sodium to a high sodium diet.** Unpublished data from a large cohort study (*n*=86) demonstrating the change in serum sodium from a low sodium diet to a high sodium diet.

1.7 Conclusions

Most studies examining the effects on dietary sodium are focused on the increase in blood pressure and negative cardiovascular consequences associated with high blood pressure. Independent of increased blood pressure, dietary sodium appears to have a profound influence on cardiovascular and vascular function. Further, elevated cardiovascular responsiveness and diseased vascular function may increase the development and progression of cardiovascular disease in normotensive

individuals. Animal studies have provided valuable insight into the robust increases in sympathetic nerve activity during chronic sodium intake and acutely elevated SNa^{+}. These studies in rats offer mechanistic approaches to how sodium sensing regions alter cardiovascular regulation during states of high SNa^{+} and osmolality. In humans, these responses have not been observed under-well controlled chronic HS conditions and compared to acute increases in sodium concentrations in normotensive individuals, and prior to the development of hypertension. Due to the overwhelming evidence associating excess dietary sodium with cardiovascular disease, future studies are needed to evaluate the effect of prolonged high dietary sodium consumption on BP regulation and determine mechanisms leading to adverse cardiovascular control.

Chapter 2

THE CHRONIC AND ACUTE EFFECTS OF HIGH SERUM SODIUM ON BLOOD PRESSURE VARIABILITY IN NORMOTENSIVE ADULTS

2.1 Introduction

Excess consumption of dietary sodium has known deleterious vascular and cardiovascular effects independent of resting blood pressure (BP) in normotensive salt-resistant (no change in mean BP) animals and humans (1, 5, 20, 23, 35, 41, 42, 51, 52). Recent evidence suggests that high sodium (HS) alters BP regulation in salt-resistant animals (1, 2, 44). The mechanism through which BP regulation is altered remains unknown. Several lines of research suggest that sodium and osmolality sensing regions within anteroventral third ventricle (AV3V) of the brain, detects excess sodium concentrations and adjust cardiovascular responses (1-3, 13, 14, 44). Compelling data in several animal studies provide evidence that consumption of HS leads to the enhanced excitability of cardiovascular control centers (1, 13, 44). The enhanced excitability is thought to be due to interaction between serum sodium (SNa^+) and the AV3V region, which then increases the excitability of the rostral ventral lateral medulla (RVLM). Animal studies have shown that enhanced excitability leads to exaggerated cardiovascular responses to sympathetic and parasympathetic stimuli (1-3, 86, 114). The exaggerated responsiveness is believed to contribute to larger fluctuations in BP and thereby increasing BP variability (86). Increased BP variability is associated with target organ damage, stroke, and future cardiovascular disease (24,

69, 83, 84, 96). Importantly, these findings suggest that HS might increase BP variability in normotensive adults independent of BP.

BP variability is considered to reflect interactions between environmental, emotional, physical activity, central (brain), and peripheral BP control mechanisms (i.e. baroreflex exercise and pressor reflex) (56). Increased BP variability has been shown to predict future cardiovascular events (24, 49) and other cardiovascular disease risks such as left ventricular hypertrophy (70), arterial stiffness (82), and microalbuminuria (97). Normotensive salt-resistant adults make up the majority of young and middle-aged adults in the United States (104), and this group regularly consumes high levels of dietary sodium (43). Therefore, determining the impact of high dietary sodium on BP variability is important for understanding health risks in a healthy population not thought to be at risk for cardiovascular disease (20, 21).

In animal studies, high SNa^+ is thought to alter BP regulation through enhanced excitability of the RVLM (86, 114). Further, recent evidence suggests that BP variability might be altered by chronic HS intake (86). Studies utilizing acute hypertonic saline infusions to increase osmolality, have found that acute increases in plasma osmolality elevate both resting sympathetic nerve activity and baroreflex control of sympathetic activity. Despite increases in sympathetic nerve activity, the increased gain of sympathetic nerve activity appears to buffer excessive increases in BP (107). However, limited data exists as to whether increasing SNa^+ chronically or acutely alters BP variability in normotensive adults. Therefore, the purpose of the following study was to determine whether chronic or acute increases SNa^+ leads to greater systolic BP variability in normotensive salt-resistant adults. We hypothesized that chronic increases in SNa^+ (i.e. 7 days of a controlled HS), but not acute increases

SNa^+ (~23 minute infusion of hypertonic saline) would increase systolic BP variability in normotensive salt-resistant adults.

2.2 Chronic High Sodium: Methods for Assessing BP Variability in Normotensive Adults

2.2.1 Study Participants

The chronic sodium loading study was a retrospective analysis of previously unanalyzed ambulatory 24h BP data from a controlled feeding study. A total of 125 participants completed the controlled feeding study. Utilizing criteria of O'Brien et al (65), participants were excluded if they did not have at least 15 daytime and 8 nocturnal BP measurements during controlled low sodium (LS) or HS diet. Participants were also excluded from analysis if blood electrolytes were not obtained during each diet or if participants were salt-sensitive (n=15; >5 mmHg Δ in mean arterial pressure low sodium to high sodium) (58). Thus, the data presented herein are the 54 participants that had an adequate number of BP measurements and blood electrolyte measurements.

All experimental protocols were approved by the Institutional Review Board at the University of Delaware in compliance with guidelines set forth by the Declaration of Helsinki. Prior to screening, all participants provided both a verbal and written consent before entering the study. During the screening visit, participants completed a health history questionnaire. A 12-lead electrocardiogram, resting BP (Dinamap, Dash 2000; GE Medical Systems, Milwaukee, WI), height, weight, waist circumference, and fasted blood sample were collected during the initial screening visit.

Participants were recruited from the area surrounding Newark, DE, USA. Participants were free of any cardiovascular disease, with no evidence of renal,

metabolic, pulmonary, or neurological diseases. Participants were non-obese (BMI < 30 kg/m^2) and did not consume tobacco products.

2.2.2 21-day Controlled Feeding Study

All food was prepared by a registered dietitian (Eugene du Pont Preventive Medicine & Rehabilitation Institute). Participants first completed a 7-day non-randomized run-in sodium diet (RS; 100 mmol Na^+/day). Immediately following the final day of the RS diet, participants completed a 7-day LS (20 mmol/day) and a 7-day HS (300mmol/day) diet in random order (See Figure 2.1). Dietary potassium was controlled across all three diets. In order to assure participants maintained a constant body weight throughout the controlled feeding study, caloric content was appropriately adjusted using the Mifflin-St Jeor equation (29). Participants were instructed to consume all of the provided food and maintain a daily fluid intake log.

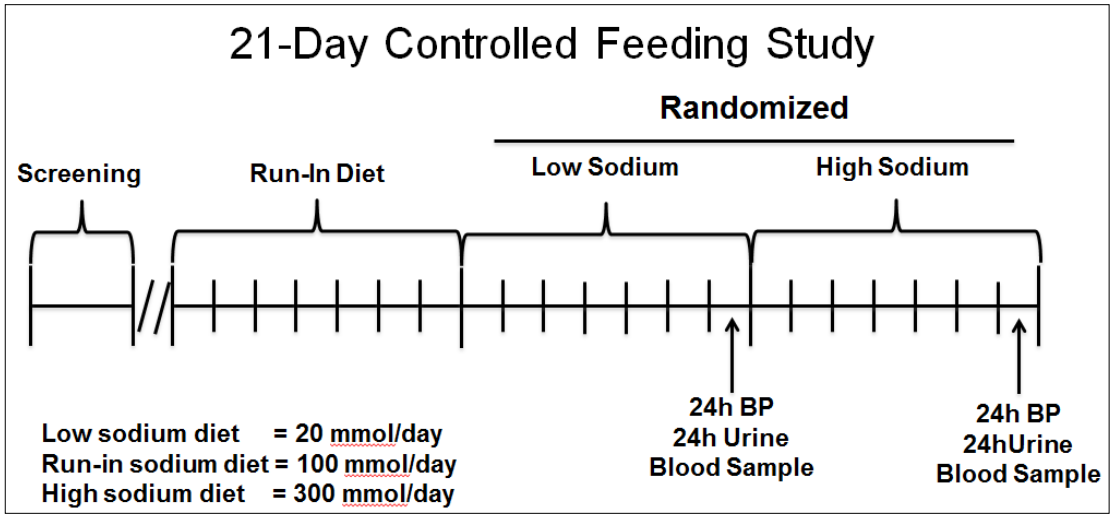

Figure 2.1 **Controlled Feeding Study.** Participants first consumed a run-in sodium diet followed by 7 days of low and high sodium in random order. Ambulatory 24h blood pressure was measured on the final day of each sodium diet.

2.2.3 Ambulatory Blood Pressure Monitoring

On the final day of each diet, participants wore a 24-hour (24h) ambulatory BP monitor (Model 90207; Spacelabs Medical, Issaquah, WA, USA). The monitors were properly fitted for each individual's arm size, as recommended by the manufacturer. Participants were instructed to maintain their normal levels of daily activity and refrain from exercise and caffeine on the final day of each diet. Participants were instructed to maintain their normal waking and sleeping patterns on the final day of each diet and monitors were set accordingly to capture wake and sleep periods. BPs were taken every 20 minutes during waking periods and every 30 minutes during sleeping periods. The relatively high frequency BP measurements allows for reliable week-to-week ambulatory BP measures, as previously demonstrated (28).

2.2.4 Urine Analysis and Blood Analysis

A fasted blood sample and 24h urine collection were obtained on the final day of each diet. Blood and urine analysis assessed electrolyte concentration under each sodium diet (EasyElectrolyte Analyzer; Medica, Bedford, MA, USA). Urinary electrolyte content was calculated and normalized to 24h. Hemoglobin (Hb 201+ model, Hemocue, Lake Forest, CA, USA) and hematocrit (Pre-Calibrated Clay Adams, Readacrit Centrifuge, Becton Dickinson, Sparks, MD, USA) were analyzed from collected whole blood samples.

2.2.5 Data & Statistical Analysis

In order for the proper assessment of systolic BP variability, all raw systolic BPs values were extracted into an Microsoft Excel Macro-Enabled spreadsheet to

calculate 24h mean BPs, 24h standard deviation (SD) of mean BPs, and 24h systolic Average Real Variability index (ARV). ARV index was derived from the following formula (61):

$$ARV = \frac{1}{N-1}\sum_{k=1}^{N-1}|BP_{k+1} - BP_k|$$

where *N* represents the number of valid BP measurements for a given time period (24h) and *BP* represents a given systolic BP measure. Both indices of BP variability have been found to have prognostic significance (39, 57); 24h SD method accounts for the total variability of BP relative to mean pressure, whereas the ARV index accounts for measurement to measurement BP variability.

BP variability was first assessed across all participants under HS and LS diets. Because the focus of the current aim was to determine the effects of increased SNa^+ on BP variability, a 2.0 mmol/L change (Δ serum sodium = HS serum sodium – LS serum sodium) in serum sodium concentration (similar to an acute sodium loading protocol described later) was utilized to divide participants into two groups: Non-Responders and Responders. Subject characteristics were compared using an unpaired two-tail *t*-test. A repeated measures ANOVA was used to evaluate the magnitude of the diet intervention on ambulatory BP variability between the two groups. The within-subjects component has two levels, LS measurements and HS measurements. Post hoc analyses were performed when necessary. Post hoc tests were performed using the Sidek's multiple comparisons test. Statistical analysis was performed utilizing GraphPad Prism 6 statistical software (GraphPad Software, Inc; La Jolla, California, U.S.A.). Values of $p<0.05$ were considered statistically different.

2.3 Acute High Sodium: Methods for Assessing BP Variability

2.3.1 Study Participants

All experimental protocols were approved by the Institutional Review Board at the University of Delaware and were in compliance with guidelines set forth by the Declaration of Helsinki. Prior to screening, all participants provided both a verbal and written consent before entering the study. During the screening visit, resting BP, height, weight, waist circumference, and a fasted blood sample was obtained.

Eighteen healthy normotensive adults (20-40 years old) participated in a controlled infusion study and 10 participated in a time control study. Participants were free of any cardiovascular disease, with no evidence of renal, metabolic, pulmonary, or neurological diseases. Participants were non-obese (BMI < 30 kg/m^2) and did not consume tobacco products.

2.3.2 Hypertonic Saline Infusion and Time Control Protocols

Three days prior to the testing visit, participants were instructed to consume ~2,300 mg Na^+/day diet and consume greater than 60 ounces of water/day. To help ensure participants were reached their 2,300mg Na^+/day goal, participants maintained a diet log accounting for portion size and sodium content of food. Participants were instructed to arrive to the testing visit fasted (6 hours), and avoid caffeine and exercise 24h prior to the study visit. All women participants were tested during the early follicular phase of the menstrual cycle or placebo phase of their oral contraceptive cycle.

Participants were rested in a comfortable supine position for the duration of the study visit. A catheter was placed in each arm, one catheter was dedicated to the infusion and the other for blood draws. Hypertonic saline (3% sodium chloride saline)

was infused for 23 minutes following a baseline measure of BP. Hypertonic saline was infused at a rate of 0.15 $ml \cdot kg^{-1} \cdot min^{-1}$ for 23 minutes. Time control participants completed a similar protocol. For time control participants, instead of receiving an infusion participants were instructed to rest quietly for 23 minutes (Figure 2.2).

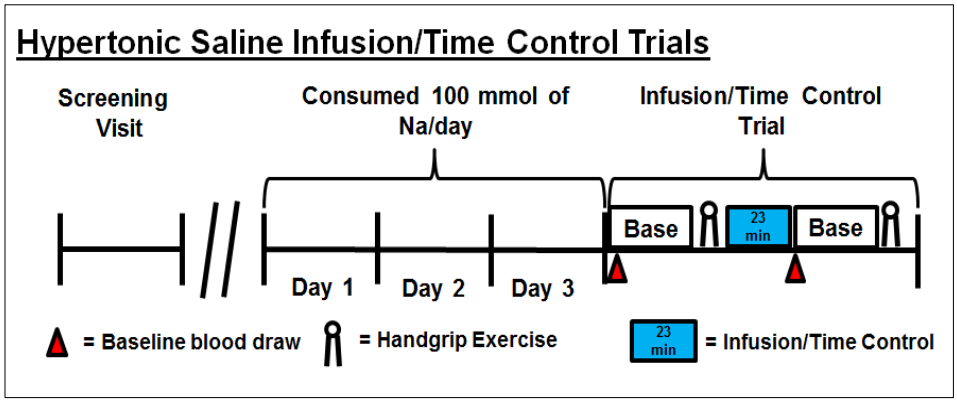

Figure 2.2 **Acute High Sodium Study Design.** We utilized two study visits to assess beat-to-beat blood pressure variability in normotensive adults under acute high sodium compared to a time control trial. Blood pressure variability was measured during each baseline period.

2.3.3 Beat-to-Beat BP

Beat-to-beat BP was measured utilizing the finger clamp volume method on the non-dominant finger (Finapres Medical Systems, Netherland) (73). The cuffs were calibrated according to manufacturer recommendations and were height adjusted to match brachial blood pressures. Beat-to-beat BPs were collected for 4.5 minutes during baseline and immediately following the 23 minute infusion. Systolic BP variability was measured by calculating beat-to-beat systolic BP SD and utilizing the ARV method.

2.3.4 Data & Statistical Analysis

Participant characteristics were compared with unpaired two-tail *t*-tests. BP variability was measured utilizing the same techniques as mentioned previously. We used paired t-tests compare pre- and post-baseline measures of BP variability under hypertonic saline and time control conditions. Post hoc tests were performed using Bonferroni method when appropriate. Statistical analysis was performed utilizing GraphPad Prism 6 statistical software (GraphPad Software, Inc; La Jolla, California, U.S.A.). Values of $p<0.05$ were considered statistically different.

2.4 Chronic High SNa^+ Results

2.4.1 Chronic High SNa^+ Study Participants and BP Variability in All Participants

Baseline characteristics can be found in Table 2.1. Twenty-four hour urine sodium excretion was significantly higher during HS compared to LS (Figure 2.3; $p<0.05$). Hemodynamic and biochemical parameters are presented in Table 2.2. HS significantly increased SNa^+, serum chloride, and plasma osmolality ($p<0.05$) compared to a LS diet. Diastolic BP was modestly increased during HS ($p<0.05$), however, both systolic BP and mean arterial pressure (Table 2.2) were not different ($p>0.05$). There was no significant difference in systolic BP variability (Figure 2.3) under HS as a group (n=54). A weak to moderate correlation (Figure 2.4) was found between the change in SNa^+ and the change in systolic BP variability ($r=0.377$, $p<0.01$).

Table 2.1 **Chronic SNa^+ Baseline Characteristics**

Baseline Characteristics	All Subjects
Number of Participants, n	n = 54
Sex	27M/27W
Age, yrs	37 ± 2
Height, cm	173 ± 1
Weight, kg	72.5 ± 1.5
BMI, kg/m2	24.1 ± 0.3
SBP, mmHg	119 ± 2
DBP, mmHg	75 ± 1
MAP, mmHg	90 ± 1

Mean ± SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

Table 2.2 **Chronic Hemodynamic and Biochemical Parameters during Low Sodium and High Sodium**

Study Parameters	Low Sodium	High Sodium
24h SBP, mmHg	115 ± 1	116 ± 1
24h DBP, mmHg	71 ± 1	70 ± 1
24h MAP, mmHg	86 ± 1	85 ± 1
Serum Sodium, mmol/l	138.3 ± 0.2	140.0 ± 0.3*
Serum Potassium, mmol/l	4.05 ± 0.05	4.00 ± 0.05
Serum Chloride, mmol/l	102.0 ± 0.2	105.5 ± 0.3*
Plasma Osmolality, mOsm/l	287 ± 0	289 ± 0*

Mean ± SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.
*$p<0.05$ (vs. Low Sodium)

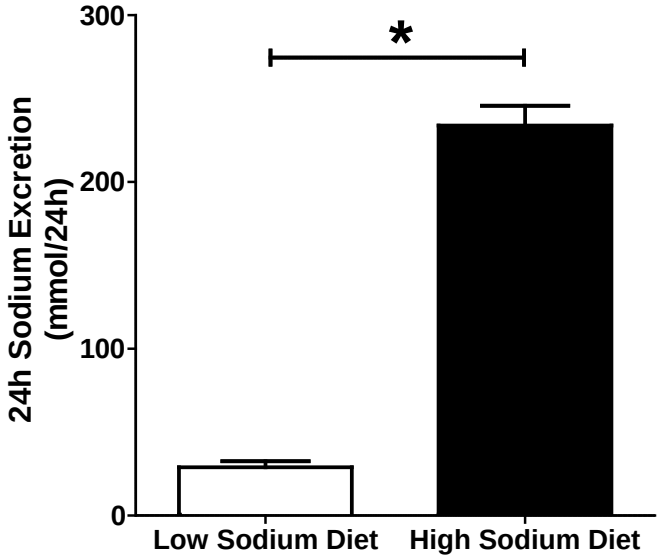

Figure 2.3 **24h Urine Sodium Excretion during Low Sodium and High Sodium Diets.** There was a significant increase in urine sodium excretion during high sodium in all participants (p<0.05).

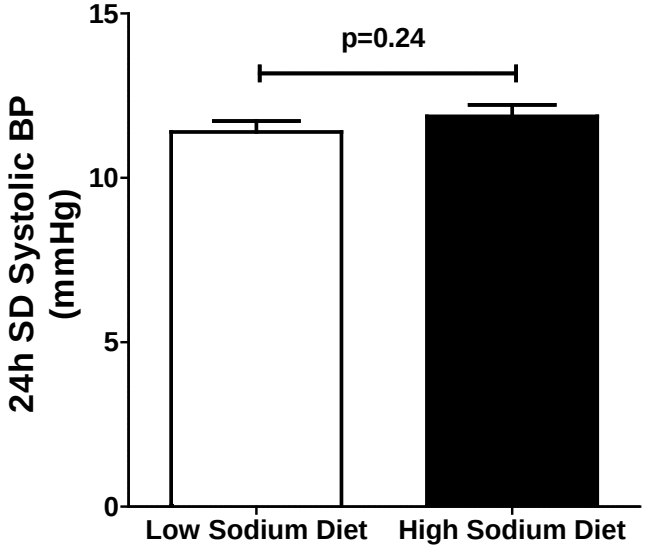

Figure 2.4 **Standard Deviation of 24h Systolic Blood Pressure in All Subjects.** Comparing the standard deviation of systolic blood pressure under LS and HS in all participants. (p=0.24 vs. Low Sodium)

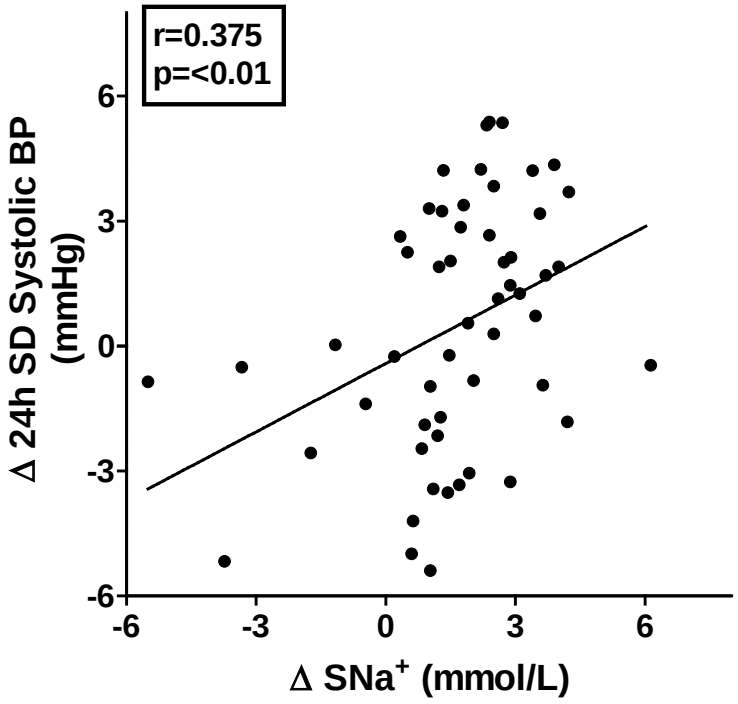

Figure 2.5 **Delta in Serum Sodium and Delta in Standard Deviation of 24h Systolic Blood Pressure.** There is a weak to moderate correlation between the change in serum sodium and the change in standard deviation of 24h systolic blood pressure (r=0.375, p<0.01).

2.4.2 Chronic Increases in SNa^+ on Systolic Blood Pressure Variability

Our participants were split into Non-Responders (<2.0 mmol/l Δ SNa^+) and Responders (>2.0 mmol/l Δ SNa^+) based on the expected change in SNa^+ under acute sodium loading conditions. Baseline characteristics can be found in Table 2.3. HS increased 24h urine sodium excretion in both groups (p<0.01), the Responders group excreted more sodium during HS compared to Non-Responders (see Figure 2.6, p<0.01). By design, our Responders group had a significant change in SNa^+ compared

to Non-Responders (ΔSNa^+ = 3.2±0.2 vs. 0.4±0.3, p<0.05, respectively). Both SNa^+ and plasma osmolality were significantly increased among Responders under HS compared to LS (main effect of diet, p<0.05), as can be seen in Table 2.4. The HS significantly increased systolic BP compared to LS, with no differences between group (main effect of diet, p<0.05).

We measured two indices of systolic BP variability between the two groups (SD & ARV). For the SD of systolic BP, there was a significant interaction (group x diet) and diet trended towards significance (p=0.08). Post hoc comparisons observed that Responders have a significant increase in systolic BP variability between LS & HS (p<0.05; Figure 2.7). For systolic ARV index, there was no significant difference between the diets or groups, however the interaction (group x diet) was significant (p<0.05; Figure 2.8). Post hoc comparisons were not significant between diets within each group.

Table 2.3 **Baseline Characteristics between Non-Responders & Responders**

Baseline Characteristics	**Non-Responders**	**Responders**
Number of Participants, n	n = 30	n = 24
Sex	15M/15W	12M/12W
Age, yrs	37 ± 3	36 ± 3
Height, cm	173 ± 1	173 ± 2
Weight, kg	72.6 ± 1.7	72 ± 2.5
BMI, kg/m2	24.1 ± 0.4	24.1 ± 0.5
SBP, mmHg	117 ± 2	123 ± 2
DBP, mmHg	75 ± 2	75 ± 1
MAP, mmHg	89 ± 2	91 ± 2

Mean ± SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

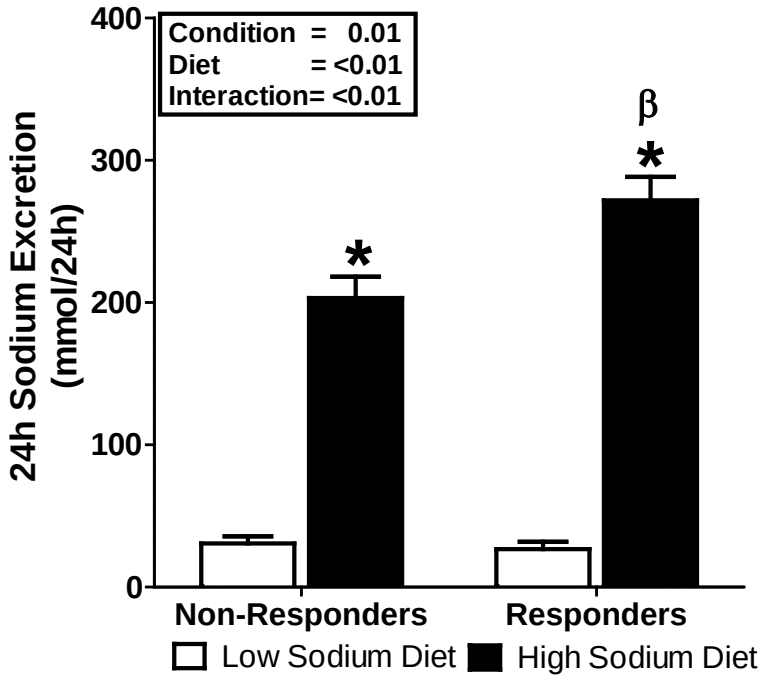

Figure 2.6 **24h Urine Sodium Excretion during Low Sodium and High Sodium Diets in Non-Responders and Responder Groups.** Urine Na^+ excretion was significantly increased during the high sodium diet in both groups(*p<0.01). The responder group excreted more sodium than the non-responder group (β p<0.01). There was no difference in 24 hour urine Na^+ excretion during low sodium.

Table 2.4 **Diet Comparisons between Non-Responders and Responders**

Measurement Variables	**Non-Responders**						**Responders**					
Diet	**Low Sodium**			**High Sodium**			**Low Sodium**			**High Sodium**		
Serum Sodium, mmol/l	138.8	±	0.3	139.2	±	0.4	137.7	±	0.3	140.9	±	0.3*†‡
Serum Potassium, mmol/l	4.06	±	0.07	3.99	±	0.07	4.04	±	0.07	4.01	±	0.07
Serum Chloride, mmol/l	101.9	±	0.3	105.4	±	0.4	102.1	±	0.4	105.52	±	0.5†
Plasma Osmolality, mOsm/l	287	±	1	288	±	1	286	±	1	289	±	1†‡
24h SD DBP, mmHg	10.5	±	0.4	9.9	±	0.4	9.6	±	0.5	10.6	±	0.4*‡
24h SD MAP, mmHg	10.2	±	0.4	10.8	±	0.5	9.6	±	0.5	9.5	±	0.5

Mean ± SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. *p<0.05 (Non-Responders vs. Responders), † p<0.05 (Low Sodium vs. High Sodium), ‡ p<0.05 (GroupxDiet).

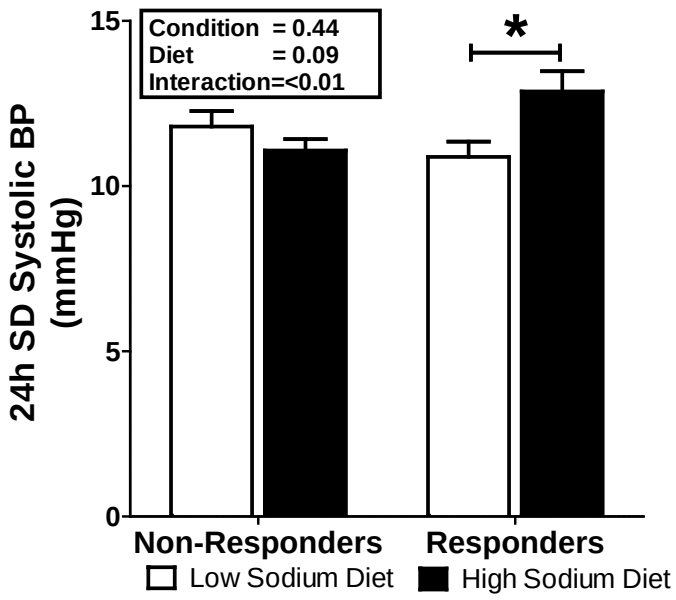

Figure 2.7 **Standard Deviation of 24h Systolic Blood Pressure.** Responders have a significant increase in BP variability from LS to HS. In those with an increase in serum sodium from low sodium to high sodium diet, 24h standard deviation of systolic BP is significantly greater under a high sodium diet compared to a low sodium diet.

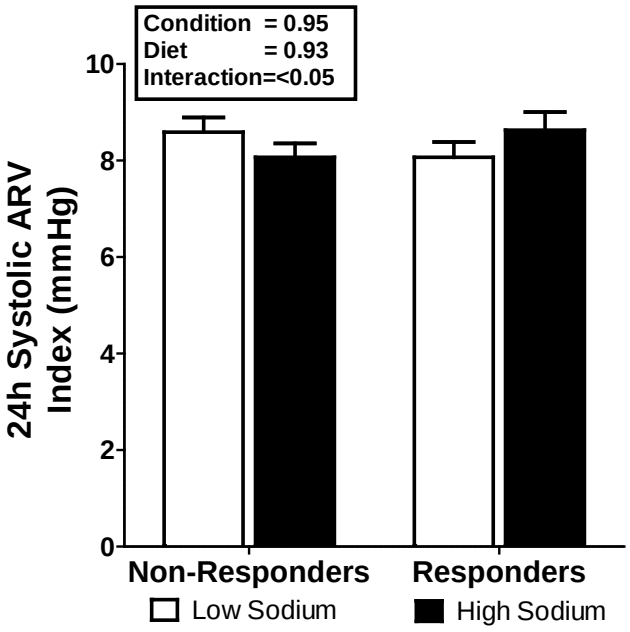

Figure 2.8 **24h Average Real Variability Index in Non-Responders and Responders.** There was significant interaction (p<0.05) between groups and diet. Post hoc comparisons were not significant between diets within each group.

2.5 Acute High SNa^+ Results

2.5.1 Study Participants

Baseline characteristics for the Hypertonic Saline infusion study can be found in Table 2.5. There were no significant differences between participants in our Hypertonic Saline trial and Time Control trial. Time Control baseline characteristic data can be found in Table 2.6.

Table 2.5 **Acute High SNa^+ Baseline Characteristics**

Screening Characteristics	**Participants**
Number of subjects	n = 18
Sex	8W/10M
Age, yr.	23 ± 1
Height, cm	171 ± 3
Weight, kg	69.7 ± 2.6
BMI, kg/m^2	23.9 ± 0.8
SBP, mmHg	117 ± 4
DBP, mmHg	70 ± 2
MAP, mmHg	81 ± 5

Mean ± SEM. W, Women; M, Men; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

Table 2.6 **Time Control Baseline Characteristics**

Screening Characteristics	**Participants**
Number of subjects	n = 10
Sex	7W/3M
Age, yr.	23 ± 1
Height, cm	167 ± 4
Weight, kg	65.3 ± 4.4
BMI, kg/m²	23.4 ± 1.3
SBP, mmHg	115 ± 4
DBP, mmHg	71 ± 3
MAP, mmHg	85 ± 3

Mean ± SEM. W, Women; M, Men;
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure;
MAP, mean arterial pressure.

2.5.2 Acute Increase in SNa^+ on BP Variability and Time Control BP Variability

By design, infusing Hypertonic Saline robustly increased SNa^+, SCl^-, plasma osmolality, and decreased hematocrit levels ($p<0.05$). We observed no changes in SNa^+, serum chloride, plasma osmolality, and hematocrit levels in our Time Control participants ($p>0.05$). There were no differences in baseline BP (see Table 2.7). We observed no significant changes in beat-to-beat SD or ARV index of systolic BP during acute high SNa^+ and Time Control (Figure 2.9, $p>0.05$).

Table 2.7 **Hemodynamic and Biochemical Parameters from Acute High SNa^+ Trial and Time Control Trial**

	Hypertonic Saline Trial		Time Control Trial	
Parameters	**Pre-Infusion**	**Post Infusion**	**Pre**	**Post**
SBP, mmHg	116 ± 3	117 ± 3	110 ± 3	112 ± 4
DBP, mmHg	66 ± 2	64 ± 2	64 ± 2	62 ± 2
MAP, mmHg	82 ± 3	82 ± 2	79 ± 2	79 ± 2
Serum Sodium, mmol/l	138.2 ± 0.3	141.3 ± 0.4*	138.9 ± 0.4	138.8 ± 0.3
Serum Potassium, mmol/l	3.95 ± 0.08	3.99 ± 0.12	3.95 ± 0.15	3.87 ± 0.12
Serum Chloride, mmol/l	103.9 ± 0.5	107.8 ± 0.6*	104.1 ± 0.4	103.8 ± 0.4
Plasma Osmolality, mOsm/l	285 ± 1	291 ± 1*	284 ± 1	284 ± 1
Hemoglobin, g/dl	12.9 ± 0.4	12.6 ± 0.4	13.1 ± 0.5	13.5 ± 0.4
Hematocrit, %	39 ± 1	38 ± 1*	39 ± 1	39 ± 2

Mean ± SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. *$p<0.05$ (pre vs. post-within trial)

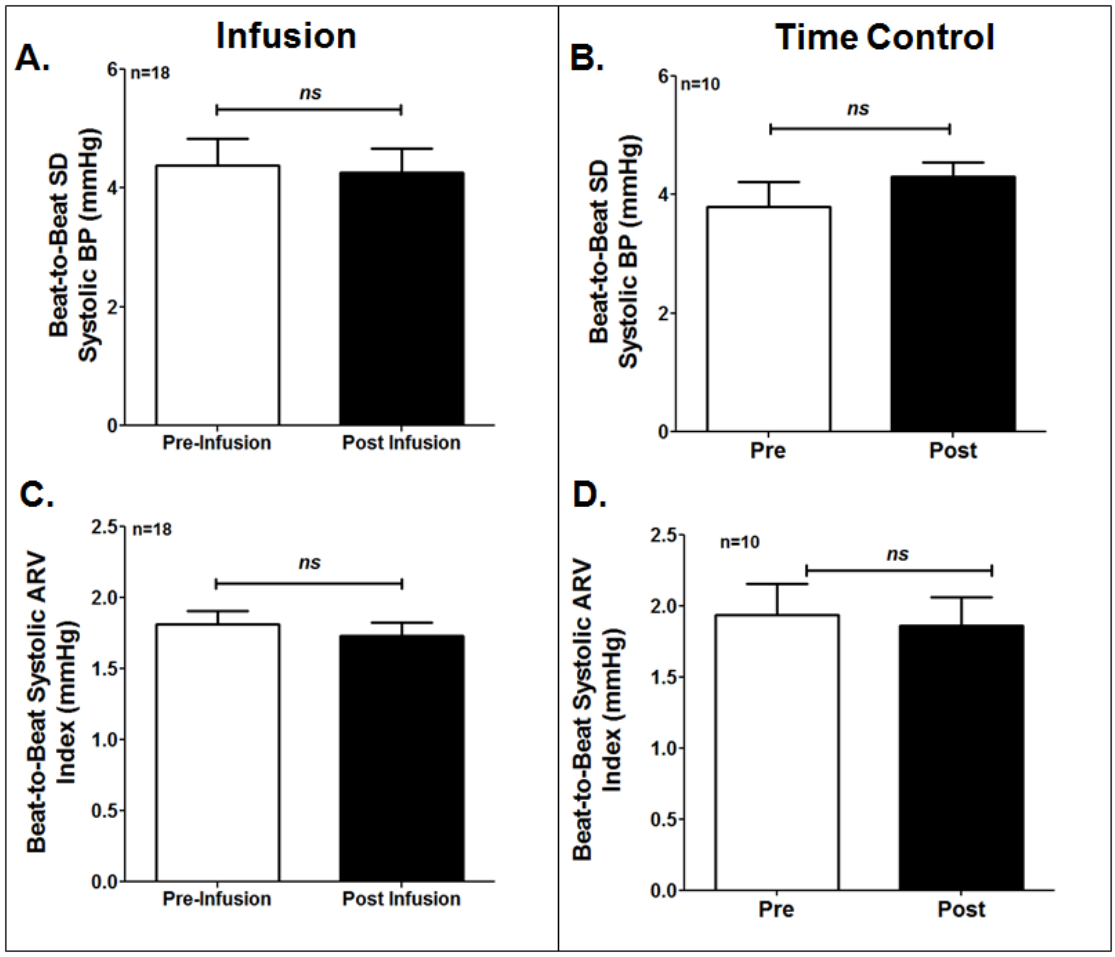

Figure 2.9 **Resting Beat-to-Beat Standard Deviation of Systolic Blood Pressure Pre & Post Hypertonic Saline Infusion.** Resting beat-to-beat blood pressure variability was not increased during acute high serum sodium conditions (p>0.05).

2.6 Discussion

Elevated BP variability is associated with target organ damage, future hypertension, cardiovascular morbidity, and mortality (18, 24, 39, 75-77, 82, 84, 96, 103). In this study, we utilized two well-controlled methods to increase SNa^+ chronically and acutely. The primary findings are that in those with chronic increases in SNa^+ (1) 24h SD of systolic BP is greater, (2) while acute high SNa^+ does not alter

beat-to-beat BP variability. These findings provide support to previous investigation in animal studies that chronic excess sodium influences 24h BP regulation independent of mean BP.

In the current study, both SD of systolic BP and ARV had similar responses in individuals with and without an increase in SNa^+, but post hoc analysis was only significant for the SD method comparing LS to HS in those with a change in SNa^+. The SD method reflects the variability of BP away from the mean, while ARV index removes the influence of mean BP and is calculated as the average absolute change in BP between consecutive measurements (61). Both SD and ARV index have prognostic significance towards predicting cardiovascular disease, target organ damage, and mortality (61, 96). Although BP variability has no defined classification for high or low BP variability in normotensive and hypertensive adults, correlative data suggests that when accounting for mean BP, individuals with the greater BP variability have increased target organ damage and cardiovascular events (84). Notably, the findings of the current study were observed in individuals classified as salt-resistant normotensives and suggest that these adults might have altered BP regulation during HS consumption when SNa^+ is chronically elevated. However, the mechanisms that increase BP variability remain unknown in humans and animals; several rodent studies suggest sodium sensing regions in the brain may influence cardiovascular regulation during prolonged HS intake.

Studies in salt-resistant animals have demonstrated that excess dietary sodium modulates BP control through the sensitization of central autonomic circuits (1, 3, 44, 86). The modulation of these circuits increases in the responsiveness to both sympathetic and parasympathetic stimuli. For instance, stimulation of

somatosympathetic reflexes in rats consuming HS results in exaggerated sympathetic nerve activity and pressor responses compared to rats fed LS. Parasympathetic mediated reflexes through vagal nerve stimulation resulted in decreased BP, heart rate, and sympathetic nerve activity (86). Although not the focus of the current study, central sensitization might influence the circadian BP pattern captured by ambulatory 24h BP monitors under chronic HS. Twenty-four hour BP variability is influenced by several external factors, including habitual activity, stress, and posture (16, 46); as well as endogenous factors such as the autonomic nervous system and the endocrine system (66, 89).

There are several other mechanisms that contribute to 24h BP variability. The circadian BP pattern captures dynamic changes in BP throughout a 24h period. Other than central sensitization, separate mechanisms influencing the circadian BP pattern include arterial and cardiopulmonary baroreflex, humoral regulation, and kidney sodium excretion (69). Increased BP variability has been associated with the development of target organ damage and future cardiovascular disease risks (24, 69, 77, 83). Given previous findings in rats fed on a chronic sodium ($\geq$14 days) (86), the current study attempted to determine whether chronic or acute increases in SNa^+ might significantly increase BP variability. The reasons for the altered circadian BP pattern found in the current study are unknown. However, these data provide evidence that even in normotensive adults, chronically increased SNa^+ elevates the standard deviation of systolic BP, which potentially increases cardiovascular disease risk in healthy salt-resistant adults.

Investigations in normotensive animals and humans have repeatedly demonstrated that an acute intravenous infusion of NaCl to raise SNa^+ activates the

sympathetic nervous system and increases BP (7, 30, 36, 92)(30, 36)(30, 36)(30, 36)(31, 37)(30, 36). Intracarotid infusions have been shown to selectively activate sodium sensing regions in the forebrain and produce similar increases in sympathetic nerve activity and BP (85). Therefore, we also measured beat-to-beat BP to determine whether acute high SNa^+ alters BP variability. Beat-to-beat BP variability assesses very short periods of BP changes, which reflects central and reflex mediated BP control, arterial stiffness, humoral fluctuations, and to a lesser extent psychological stress (69, 103). Previous studies have observed acute infusions of NaCl increases baroreflex function in healthy adults along with increases in muscle sympathetic nerve activity and BP (107). In the current study, beat-to-beat BP variability remained unaltered during acutely increased SNa^+. Increased baroreflex gain might have prevented increases in BP variability during acute high SNa^+. In healthy adults, normal baroreflex function works to buffer acute exaggerations in BP; however baroreflex buffering capacity declines with aging and in hypertension, leading to increased BP variability (63). Although, BP variability remained unchanged during acute conditions, the long term influence of elevated SNa^+ on sympathetic outflow remains unknown.

Limitations

There are several limitations to the current findings. During the chronic condition, participants were tested during varying phases of the menstrual cycle, and menopausal status was also unaccounted for among the cohort of healthy normotensive women. A previous study in healthy normotensive women found that the menstrual cycle had no effect on 24h BP during LS and HS diets (72). Another limitation to the current study was the inability to address individuals for salt-

sensitivity under acute sodium conditions. Previous studies assessing salt-sensitivity have utilized larger sample sizes and protocols using restrictive sodium intake and saline infusions coupled by furosemide (diuretic) administration following sodium/volume depletion (95, 106). Weinberger et al (105) suggested only 26% of normotensive adults are salt-sensitive, which under HS conditions, typically results in elevated BP. Although unable to exclude for salt-sensitivity status under acute HS conditions, it is unlikely that salt-sensitivity influenced these findings because mean BP and BP variability were unchanged in our study population. Lastly, two measures of BP (24h & beat-to-beat) were assessed during chronic and acute conditions. Therefore, the findings of the current study can only be generalized within each respective study design. Future studies are still needed to determine whether chronic increases in SNa^+ augment beat-to-beat BP variability.

2.7 Conclusion

In conclusion, chronic but not acute increased SNa^+ leads to increased 24h systolic BP variability in normotensive salt-resistant adults. The mechanisms that increase BP variability during chronic high SNa^+ remain unknown. Future studies are needed to determine the pathways through which chronically elevated SNa^+ leads to greater systolic BP variability in normotensive adults.

Chapter 3

THE EFFECTS OF CHRONIC AND ACUTE INCREASES IN SODIUM ON BLOOD PRESSURE RESPONSES DURING HANDGRIP EXERCISE

3.1 Introduction

In the United States, adults consume roughly 48 mmol of Na^+/day over the recommended 100 mmol of $Na^{+/}$day (111). The consumption of high dietary sodium negatively influences BP regulation in hypertensives, chronic kidney disease, and hyperaldosteronism (31, 99-101). Recent animal studies have shown high sodium (HS) alters blood pressure (BP) regulation in normotensive rat models, providing further evidence of potential cardiovascular risks in humans related to dietary sodium consumption (44, 86, 114).

Yamauchi et al (114), found that normotensive rats (Spraque-Dawley) consuming HS for 14 to 15 days had exaggerated BP responses to exercise pressor reflex activation. Many studies have suggested that the exaggerated cardiovascular responses are enhanced through neuronally-mediated mechanisms that sense high serum sodium (SNa^+) (44, 71, 85, 114). Chronic adaptations augmenting cardiovascular responses are believed to be due to slowly-developing changes in neuronal plasticity within the rostral ventrolateral medulla (RVLM) (92). In studies acutely increasing SNa^+ in both animals and normotensive adults, acute increases in SNa^+ augmented sympathetic nerve activity and mean arterial BP (13, 36). These responses are generally thought to be mediated by sodium sensing regions in the

anteroventral third ventricle of the brain (85). However acutely increasing SNa+ likely does not alter neuronal plasticity.

In healthy normotensive adults, HS is over consumed in most American diets (62). Excess consumption of sodium has been shown to have negative cardiovascular effects independent of BP (5, 6, 20, 23, 35). Limited data exists as to whether chronic or acute increases in SNa^+ influences BP responses to exercise. Data in borderline hypertensives observed decreased BP responses to exercise during sodium restriction compared to habitual HS intake (5). Importantly, exaggerated BP responses to exercise has been shown to predict the future incidence of hypertension in healthy normotensives (59). However, no studies in normotensive adults have utilized two well-controlled methods to chronically and acutely increase SNa^+ and measure BP responses to exercise pressor reflex activation. Therefore, in light of recent findings suggesting normotensive rats have exaggerated BP responses to exercise pressor reflex activation (114), the purpose of the following study was to determine whether chronic and/or acute increases in SNa^+ leads to exaggerated BP responses in normotensive adults. The hypothesis was that chronic, but not acute increases in SNa^+ would increase BP responses to the exercise pressor reflex.

3.2 Chronic Increases in SNa^+ Methods

3.2.1 Study Population

Eighteen normotensive participants (age 22-59 yrs) were recruited from the population surrounding Newark, DE, USA. All participants were free of hypertension, cardiovascular disease, diabetes, obesity (BMI > 30 kg/m^2), chronic kidney disease,

metabolic disorders and cancers. Participants were excluded if they used nicotine products.

3.2.2 21-Day Controlled Feeding Study

Participants completed a 21-day controlled feeding study. Food was prepared by a registered dietitian (Eugene du Pont Preventive Medicine & Rehabilitation Institute). Participants first completed a 7-day standardized run-in diet (RS; 100 mmol sodium/day). Immediately following the final day of the standardized run-in diet, participants completed a 7-days of low sodium (LS; 20 mmol/day) and HS (300 mmol/day) diets in random order (Figure 3.1). Dietary potassium was controlled across all three diets. In order to ensure participants maintained a constant body weight throughout the controlled feeding study, energy content was appropriately adjusted using the Mifflin-St Jeor equation (29). Participants were instructed to consume all of the food provided.

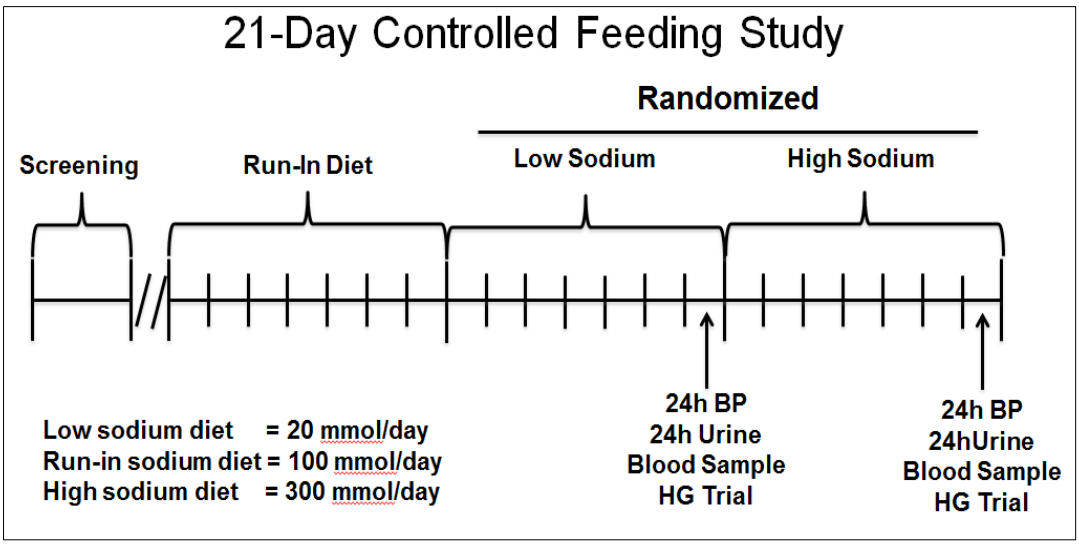

Figure 3.1 **Controlled Feeding Study Design.** Participants first consumed a 7-day run-in diet followed by 7 days of low and high sodium in random order. On the final day of each diet, we measured ambulatory 24h blood pressure, a 24h urine collection, a fasted blood sample, and blood pressure responses to handgrip exercise followed by post exercise ischemia.

3.2.3 Assessment of Blood Pressure Responses to Handgrip Exercise

On the final day of each diet, participants reported to the lab fasted, and instructed to avoid exercise and caffeine 24 hours (24h) prior to the study visit. Participants were tested in a comfortable recumbent position with the head supported above the plane of the body. Beat-to-beat BP was measured using a Finometer (Finger volume clamp method- Finapres Medical Systems, Netherland) (73) calibrated to brachial BPs according to the manufacturer's recommended calibration procedures. Brachial BPs were measured by automated sphygmomanometer (Dinamap Dash 2000, GE Medical Systems, WI) to verify Finometer BPs. Heartrate was obtained by six lead ECG (Dinamap Dash 2000, GE Medical Systems, WI). Respiratory measurements

were obtained by strain-gauge pneumograph (Pneumotrace; UFI, CA) placed around the abdomen to ensure participants did not inadvertently perform a Valsalva during exercise. Rating of perceived exertion (RPE) was measured during exercise using the 6-20 Borg scale, providing a relative index of central command's influence during exercise (9).

Participants performed isometric handgrip exercise with their dominant arm at 40% of maximal voluntary contraction (MVC) for 2 minutes. In order to isolate the metaboreflex of the exercise pressor reflex, post-exercise ischemia (PEI) was performed by rapidly inflating an occlusion cuff immediately following handgrip exercise (Hokanson, Inc, Bellevue, WA, USA) to 250 mmHg on the dominant arm for 3 minutes, followed by a recovery period (Figure 3.2). Responses to handgrip exercise and PEI were reported as the absolute BP response and changes from baseline during each diet.

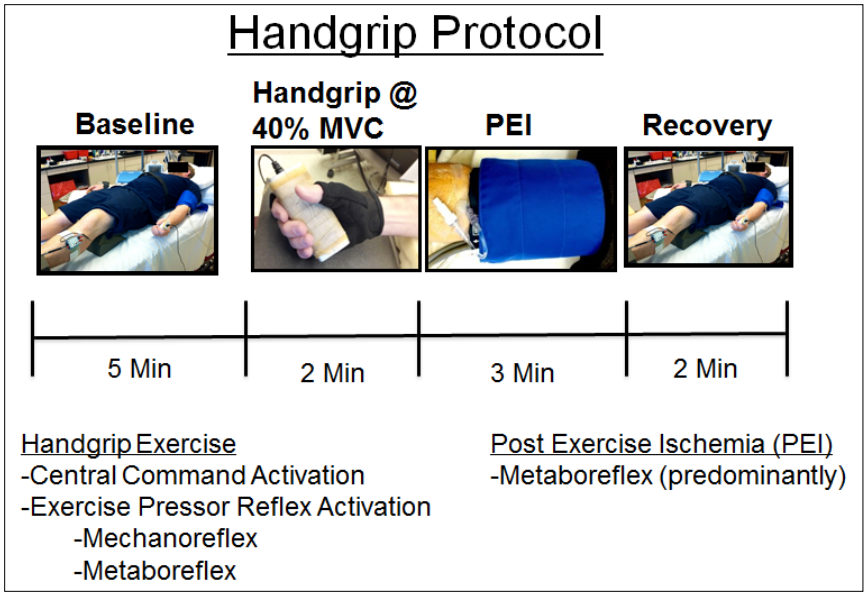

Figure 3.2 **Handgrip Protocol.** Protocol utilized to measure blood pressure responses to exercise pressor reflex activation.

3.2.4 Blood, Urine Analysis, and Ambulatory Blood Pressure

A fasted blood sample and 24h urine collection were obtained on the final day of each diet. Blood and urine were analyzed for electrolyte concentration (EasyElectrolyte Analyzer; Medica, Bedford, MA, USA). Urinary electrolyte content was calculated and normalized to 24h. Osmolality was measured from blood plasma (Advanced 3d3 Osmometer; Advanced Instruments, Norwood, MA, USA). Hemoglobin (Hb 201+ model, Hemocue, Lake Forest, CA, USA) and hematocrit (Pre-Calibrated Clay Adams, Readacrit Centrifuge, Becton Dickinson, Sparks, MD, USA) were analyzed from collected whole blood samples. On the final day of each diet, participants wore a 24h ambulatory BP monitor (Model 90207; Spacelabs Medical, Issaquah, WA, USA) set to measure BP every 20 minutes during daytime periods and every 30 minutes during nocturnal periods. BP data obtained from the 24h BP monitor was used to assess salt-sensitivity, as described later.

3.2.5 Statistical Analysis & Salt-Sensitivity Assessment

Participant biochemical parameters were compared utilizing paired *t*-tests between the two diet conditions. A repeated-measures ANOVA was performed to determine the effect of chronic high SNa^{+} on exercise and metaboreflex isolation. Post hoc tests were performed using Sidak's multiple comparison test. Statistical analysis was performed using GraphPad Prism 6 statistical software (GraphPad Software, Inc; La Jolla, California, U.S.A.). Participants with >5 mmHg increase in 24h mean arterial pressure from the LS to HS diet were classified as salt-sensitive and excluded from analysis (58).

3.3 Acute High SNa^+ Methods

3.3.1 Study Population

Twenty-six normotensive participants (age 20-40 yrs) were recruited from the population surrounding Newark, DE, USA. All participants were free of hypertension, cardiovascular disease, diabetes, obesity (BMI > 30 kg/m^2), chronic kidney disease, metabolic disorders and cancers. Nicotine consumers were excluded from the study. Prior to testing, participants attempted to consume a 100 mmol sodium/day, maintain ad libitum hydration and recorded a 3 day diet log. The morning following the completion of the 3 day 100 mmol sodium/day diet, participants reported to the Cardiovascular Physiology Laboratory at the University of Delaware.

3.3.2 Hypertonic Saline Infusion and Time Control Trials

Participants were instructed to arrive to the testing visited fasted (6 hours), avoid caffeine and exercise 24h prior to the study visit. All participants were rested in a comfortable supine position for the duration of the study visit. A catheter was placed in the antecubital vein of each arm, one catheter was dedicated to the infusion and the other for blood draws. Hypertonic saline (513 mEq/L) was infused for 23 minutes following a baseline measure of resting BP and muscle sympathetic nerve activity. The rate of hypertonic saline infusion was 0.15 $ml \cdot kg^{-1} \cdot min^{-1}$ for 23 minutes as used previously (36), and is similar to the increase in SNa^+ during controlled HS. Time control participants completed a similar protocol. Instead of receiving an infusion, time control participants were instructed to rest quietly for 23 minutes. Two participants completed both hypertonic saline infusion and time control protocols.

Blood draws were performed at baseline and during the final minute of PEI of the first handgrip trial and immediately following the 23 minute infusion/quiet rest for

post baseline and during the post infusion/quiet rest handgrip trial. The blood draws allowed for the assessment of serum electrolyte content (EasyElectrolyte Analyzer; Medica, Bedford, MA, USA), blood lactate (Lactate Plus Analyzer, Nova Biomedical, Waltham, MA), blood pH (AB15 pH Meter, Fisher Scientific, U.S.A). Plasma osmolality (Advanced 3d3 Osmometer; Advanced Instruments, Norwood, MA, USA), hemoglobin (Hb 201+ model, Hemocue, Lake Forest, CA, USA), and hematocrit (Pre-Calibrated Clay Adams, Readacrit Centrifuge, Becton Dickinson, Sparks, MD, USA) were measured only at the baseline pre- and post-infusion/quiet rest.

3.3.3 Assessment of Blood Pressure and Muscle Sympathetic Nerve Activity during Handgrip Exercise

Participants were tested in a recumbent position with their head and one leg supported just above the plane of the body. Beat-to-beat BP, brachial BPs, heart rate, respiration, and RPE were collected as previously described. The protocol is provided in Figure 3.3.

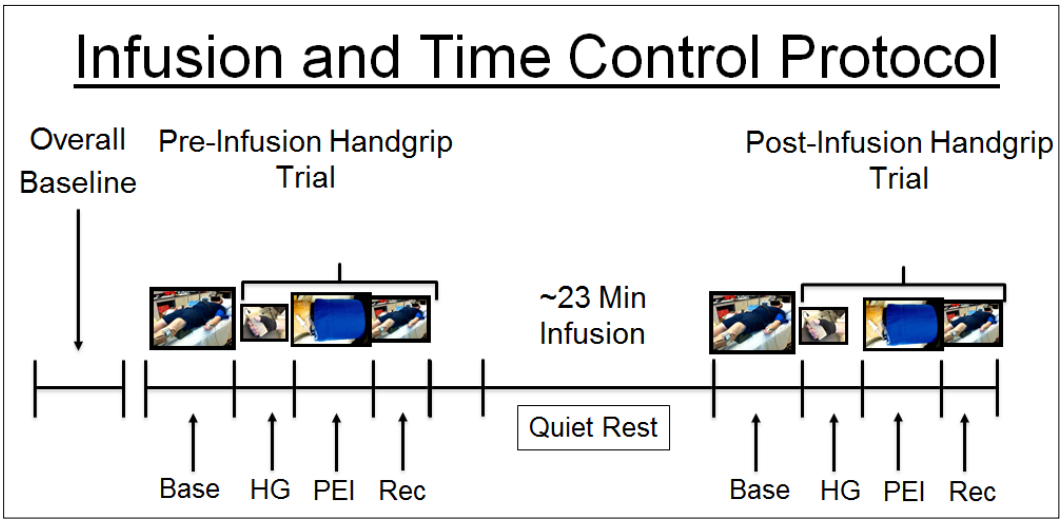

Figure 3.3 **Hypertonic Saline and Time Control Trial Protocol.** Protocol utilized to test whether acute increases in SNa+ increases exercise pressor reflex activation

Microneurography was utilized to assess muscle sympathetic nerve activity throughout the testing protocol, to determine the role of the sympathetic nervous system during acute high SNa^{+} and time control trial. A primary tungsten recording microelectrode was inserted in the peroneal nerve behind the fibular head and a reference microelectrode was inserted 2-3 cm from the primary microelectrode, as previously performed in our lab (36). The nerve recording was amplified (factor = 70,000), bandpass filtered (700-2,000 Hz), rectified, and integrated (time constant 0.1s) using a nerve traffic analyzer (Nerve Traffic Analyzer, model 662c-3; University of Iowa, Bioengineering, Iowa City, IA, USA). An adequate nerve recording was confirmed prior to the experimental protocols utilizing the following criterion: absence of afferent nerve activity during light skin stroking, increased efferent nerve activity during voluntary end-expiratory apnea, and a visual 3 to 1 signal to noise ratio of

spontaneous cardiac cycle gating of efferent nerve bursts. Offline analysis of MSNA was performed utilizing a customized Labview program (25), which provides beat-to-beat synchronization of heart rate, MSNA, and BP. The program employs R-wave gating to detect MSNA bursts (1.2±0.3s from initial R-wave) and all bursts were visually confirmed by a trained microneurographer. The highest three detected bursts were averaged and assigned a value of 100 arbitrary units (AU); and all bursts were scaled accordingly.

3.4 Chronic High SNa^+ Results

3.4.1 Chronic High Sodium Loading Participants

Two participants were classified as salt-sensitive and removed from analysis. Therefore, the remaining 16 subjects are presented. Baseline characteristics are presented in Table 3.1. All participants were normotensive with normal BMI. Metabolic, kidney, and liver function were all within normal limits.

Table 3.1 **Chronic Sodium Diet Screening Characteristics**

Screening Characteristics	Participants
Number of Participants, n	n = 16
Sex	8F / 8M
Age, yrs	39 ± 4
Height, cm	170 ± 2
Weight, kg	67.8 ± 2.4
BMI, kg/m2	23.3 ± 0.5
SBP, mmHg	117 ± 3
DBP, mmHg	71 ± 2
MAP, mmHg	87 ± 2

Mean ± SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

3.4.2 Blood Pressure Responses during Handgrip Exercise under Chronic High Sodium

Twenty-four hour hemodynamic and biochemical parameters during the final day of the randomized LS & HS sodium diets are presented in Table 3.2. Urine sodium excretion was significantly higher during HS compared to LS ($p<0.05$). There was no difference in BP between LS and HS diets ($p>0.05$). Both SNa^+ and plasma osmolality were significantly increased during the HS diet ($p<0.05$). Hematocrit was significantly decreased during HS compared to LS ($p<0.05$), suggesting plasma volume expansion.

Baseline hemodynamic data prior to the start of HG exercise are presented in Table 3.3. Baseline HR was lower during HS compared to LS ($p<0.05$). All other hemodynamic data was not significantly different during LS & HS ($p>0.05$). Compared to baseline systolic BP, there was a significant effect of exercise (See Figure 3.2, $p<0.05$) and significant interaction ($p<0.05$). We performed post hoc

analyses to determine the interaction. The post hoc analysis revealed that during chronic HS, systolic BP was significantly increased at baseline & during PEI. There was no significant change from baseline during HG exercise between diets (Figure 3.3; $p>0.05$); however, the change from baseline during PEI was significantly higher during HS compared to LS (Figure 3.4; $p<0.05$). Interestingly, post hoc analysis revealed that mean arterial pressure was higher during LS compared to HS ($p<0.05$); however the change from baseline was not significantly different than HS (Figure 3.5). All other hemodynamics measured during handgrip exercise and PEI can be seen in Table 3.4.

Table 3.2 **Hemodynamic & Biochemical Parameters under Controlled Sodium Diets**

Randomized Sodium Diet	**Low Sodium**	**High Sodium**
Number of Participants, n	n = 16	n = 16
24h SBP, mmHg	115 ± 2	113 ± 2
24h DBP, mmHg	70 ± 1	67 ± 1
24h MAP, mmHg	85 ± 1	83 ± 2
Serum Sodium, mmol/l	137.4 ± 0.6	140.0 ± 0.4*
Serum Potassium, mmo/l	3.96 ± 0.11	4.08 ± 0.14
Serum Chloride, mmol/l	102.3 ± 0.6	106.2 ± 0.8*
Osmolality, mOsm/kg H_2O	285 ± 1	289 ± 1*
Hematocrit, %	42 ± 1	40 ± 1*
Hemoglobin, mg/dl	13.4 ± 0.4	12.7 ± 0.4
24h Urine Sodium, mmol/24h	30.4 ± 4.6	279.2 ± 16.3*

Mean ± SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.
*p<0.05 (Low Sodium vs. High Sodium)

Table 3.3 **Baseline Hemodynamic Parameters under Chronic Sodium**

Baseline Parameters	**Low Sodium**	**High Sodium**
SBP, mmHg	113 ± 3	116 ± 4
DBP, mmHg	58 ± 3	56 ± 3
MAP, mmHg	77 ± 3	75 ± 3
Heart Rate, beats/min	61 ± 2	58 ± 2*
Cardiac Output, L/min	5.24 ± 0.23	5.74 ± 0.28
Total Peripheral Resistance, cgs	1487 ± 96	1401 ± 77

Mean ± SEM. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. *p<0.05 (Low Sodium vs. High Sodium)

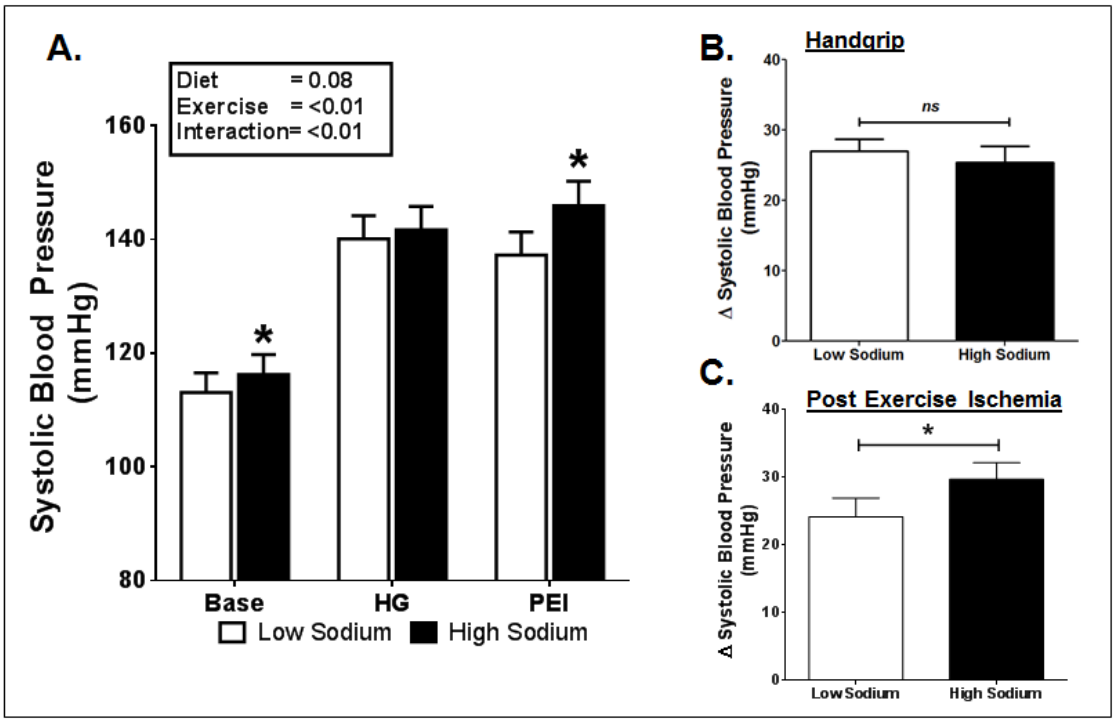

Figure 3.4 **Systolic Blood Pressure during the Chronic Sodium Handgrip Trial.** Systolic blood pressure (A) at baseline (Base), during handgrip exercise (HG) and post exercise ischemia (PEI). During Base and PEI, systolic blood pressure was significantly increased compared to Low Sodium. Indexed to baseline, there was no change in systolic BP response during HG (B; $p>0.05$), PEI systolic BP response was significantly increased relative to baseline (C; $p<0.05$)

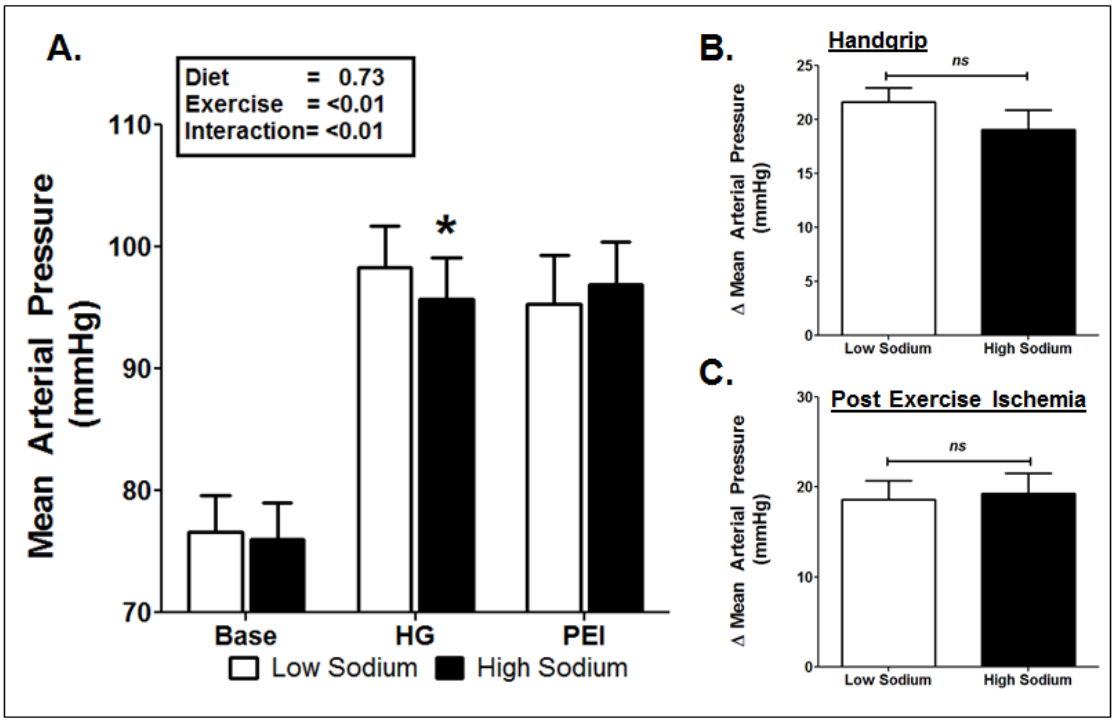

Figure 3.5 **Mean Arterial Pressure Response to Handgrip Exercise and Post Exercise Ischemia.** Mean arterial blood pressure (A) at baseline, during handgrip exercise (HG) and post exercise ischemia (PEI). There was a significant difference between low sodium and high sodium during HG (post hoc, $p<0.05$). Indexed to baseline, there was no change in mean arterial blood pressure response during HG (B) and PEI (C) ($p>0.05$).

Table 3.4 **Hemodynamics during Handgrip Exercise and Post Exercise Ischemia**

Hemodynamic Parameters	**Low Sodium**						**High Sodium**					
Trial Phase	**HG**			**PEI**			**HG**			**PEI**		
DBP, mmHg	77	±	3	74	±	4	73	±	3	72	±	3
MAP, mmHg	98	±	3	95	±	4	96	±	3	97	±	3
Heart Rate, beats/min	77	±	3	64	±	2	78	±	3	62	±	3
Cardiac Output, L/min	6.24	±	0.38	5.84	±	0.31	7.77	±	0.46*	6.67	±	0.35*
TPR, cgs	1605	±	122	1679	±	158	1319	±	103*	1537	±	92

Mean ± SEM. HG, handgrip exercise; PEI, post exercise ischemia; DBP, diastolic blood pressure; MAP, mean arterial pressure; TPR, total peripheral resistance; cgs, centimeter-grams/second. *$p<0.05$ (vs. Low Sodium)

3.5 Acute High SNa^+ Results

3.5.1 Acute Sodium Loading and Time Control Participants

Baseline characteristics for participants enrolled into the acute sodium loading trial are presented in Table 3.5. All participants were healthy with normotensive BP and non-obese. All participants had both healthy liver and kidney function within normal limits. All baseline Time Control participant data can be found in Table 3.6. There were no significant differences between participants completing the acute sodium loading trial and Time Control trial.

Table 3.5 **Screening Characteristics from the Acute High SNa^+ Trial**

Screening Characteristics	Participants
Number of subjects	18
Sex	8W/10M
Age, yr.	23 ± 1
Height, cm	171 ± 3
Weight, kg	69.7 ± 2.6
BMI, kg/m²	23.9 ± 0.8
SBP, mmHg	117 ± 4
DBP, mmHg	70 ± 2
MAP, mmHg	81 ± 5

Mean ± SEM. W, Women; M, Men; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

Table 3.6 **Screening Characteristics from the Time Control Trial**

Screening Characteristics	Participants
Number of subjects	10
Sex	7W/3M
Age, yr.	23 ± 1
Height, cm	167 ± 4
Weight, kg	65.3 ± 4.4
BMI, kg/m²	23.4 ± 1.3
SBP, mmHg	115 ± 4
DBP, mmHg	71 ± 3
MAP, mmHg	85 ± 3

Mean ± SEM. W, Women; M, Men; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

3.5.2 Blood Pressure and Muscle Sympathetic Nerve Activity Responses during Handgrip Exercise and Post Exercise Ischemia

Hypertonic saline increased SNa^+, and plasma osmolality (Figure 3.5; $p<0.05$ vs. pre-infusion baseline), while decreasing hematocrit ($p<0.05$ vs. pre-infusion baseline). The acute infusion of hypertonic saline did not increase BP ($p>0.05$), however there was a significant increase in cardiac output and total peripheral resistance ($p<0.05$) compared to pre-infusion baseline.

Acute increases in SNa^+ had no effect on BP (systolic & mean arterial pressure) responses during handgrip exercise and PEI (Figure 3.5). BP responses relative to baseline were not different during HG (Figure 3.6) or PEI (Figure 3.7). However, acute increases in SNa^+ did significantly increase baseline muscle sympathetic burst frequency, which remained elevated during handgrip exercise and PEI (n=13, main effect of SNa^+, $p<0.05$). Additionally, there was a main effect ($p<0.05$) of high SNa^+ on Total MSNA post-infusion (Figure 3.8). Relative to baseline there was a significant increase in sympathetic nerve activity during handgrip exercise (Figure 3.9, $p<0.05$), but no significant increase during PEI (Figure 3.10, $p>0.05$). During PEI, blood lactate was significantly increased from baseline pre- and post-infusion (main effect of time, $p<0.05$), but was not different baseline (Pre= 0.9±0.1 mmol/L vs. Post= 0.9±0.1 mmol/L) or during PEI (Pre PEI= 3.0±0.2 mmol/L vs. Post PEI= 2.7±0.2 mmol/L). Similarly blood pH during PEI was significantly increased from baseline pre- and post-infusion (main effect of time, $p<0.05$), but was not different baseline (Pre= 7.47±0.02 vs. Post= 7.45±0.02, $p>0.05$) or during PEI (Pre PEI= 7.42±0.03 vs. Post PEI= 7.40±0.03, $p>0.05$).

During the Time Control trial, there were no significant changes from baseline for all measured biochemical parameters. All baseline biochemical parameters (pre vs.

post) are presented in Table 3.7. Pre to post baseline hemodynamics were not different (p>0.05). All baseline hemodynamic parameters for both trials are presented in Table 3.8. There was no change in muscle sympathetic nerve activity during the Time Control trial (n=6, p>0.05). All muscle sympathetic nerve activity parameters are provided in Table 3.9. During the Time Control trial, there was no significant increase in BP or muscle sympathetic nerve activity, which are presented in Figures 3.5, 3.6, 3.7, 3.8, 3.9, 3.10 (p>0.05). During PEI, blood lactate was significantly increased from baseline pre- and post-infusion (main effect of time, p<0.05), but was not different at baseline (Pre= 0.9±0.1 mmol/L vs. Post= 0.9±0.1 mmol/L) or during PEI (Pre PEI= 3.0±0.2 mmol/L vs. Post PEI= 2.7±0.2 mmol/L). Similarly blood pH during PEI was significantly increased from baseline pre- and post-infusion (main effect of time, p<0.05), but was not different baseline (Pre= 7.57±0.03 vs. Post= 7.57±0.04, p>0.05) or during PEI (Pre PEI= 7.50±0.04 vs. Post PEI= 7.48±0.05, p>0.05).

Table 3.7 **Baseline Biochemical Parameters during Acute High SNa^+ and Time Controls**

	Hypertonic Saline		Time Control	
Biochemical Parameters	**Pre-Infusion**	**Post Infusion**	**Pre**	**Post**
Serum Sodium, mmol/l	138.2 ± 0.3	141.3 ± 0.4*	138.9 ± 0.4	138.8 ± 0.3
Serum Potassium, mmol/l	3.95 ± 0.08	3.99 ± 0.12	3.95 ± 0.15	3.87 ± 0.12
Serum Chloride, mmol/l	103.9 ± 0.5	107.8 ± 0.6*	104.1 ± 0.4	103.8 ± 0.4
Plasma Osmolality, mOsm/l	285 ± 1	291 ± 1*	284 ± 1	284 ± 1
Hemoglobin, g/dl	12.9 ± 0.4	12.6 ± 0.4	13.1 ± 0.5	13.5 ± 0.4
Hematocrit, %	39 ± 1	38 ± 1*	39 ± 1	39 ± 2

Mean ± SE. *p<0.05 (pre vs. post, within trial)

Table 3.8 **Baseline Hemodynamic Parameters during Acute High SNa^+ and Time Controls**

	Hypertonic Saline Trial		Time Control Trial	
Haemodynamic Parameters	**Pre-Infusion**	**Post Infusion**	**Pre**	**Post**
SBP, mmHg	116 ± 3	117 ± 3	110 ± 3	112 ± 4
DBP, mmHg	66 ± 2	64 ± 2	64 ± 2	62 ± 2
MAP, mmHg	82 ± 3	82 ± 2	79 ± 2	79 ± 2
Heartrate, beats/min	58 ± 2	60 ± 2	60 ± 5	62 ± 4
Cardiac Output, L/min	5.22 ± 0.25	5.75 ± 0.22*	4.70 ± 0.70	4.76 ± 0.64
TPR, cgs	1572 ± 81	1459 ± 58*	1750 ± 292	1646 ± 184

Mean ± SE. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; TPR, Total Peripheral Resistance; cgs, centimeter-grams/second. *p<0.05 (pre vs. post, within trial)

3.9 **Baseline Muscle Sympathetic Nerve Activity during Acute High SNa^+ and Time Controls**

	Hypertonic Saline		Time Controls	
MSNA Parameters	**Pre-Infusion**	**Post Infusion**	**Pre**	**Post**
Number of Participants, n	n = 13	n = 13	n = 6	n = 6
Burst Frequency, bursts/min	11 ± 2	15 ± 2*	7 ± 2	8 ± 3
Burst Incidence, bursts/100beats	20 ± 3	26 ± 4	16 ± 5	19 ± 5
Total MSNA, AU/beat	9 ± 2	11 ± 2	7 ± 2	8 ± 3
Total Activity, AU/min	526 ± 105	634 ± 100	378 ± 78	462 ± 113

Mean ± SE. MSNA, muscle sympathetic nerve activity; AU, arbitrary units. *p<0.05 (pre vs. post, within trial)

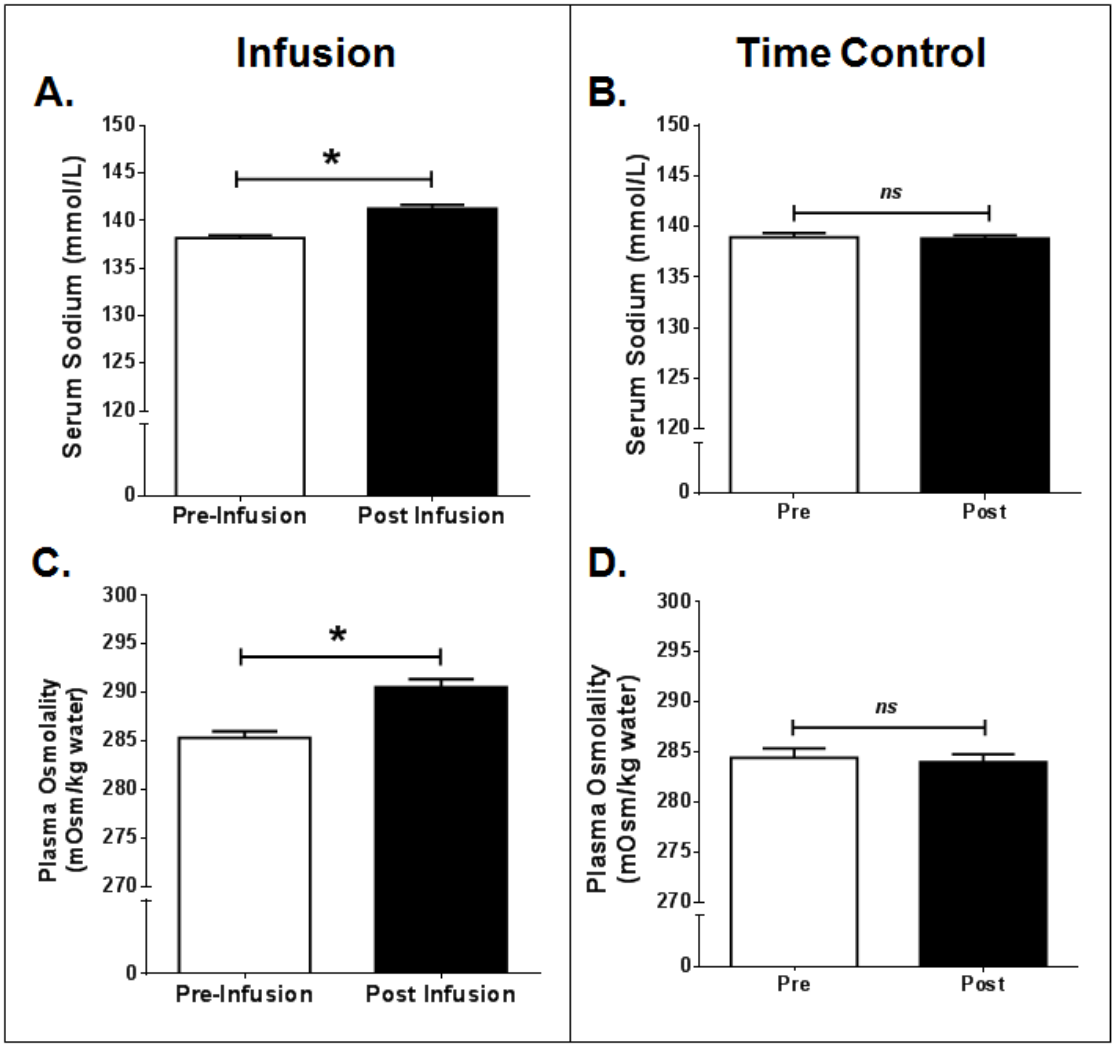

Figure 3.6 **Hypertonic Saline Significantly Increases Serum Sodium and Plasma Osmolality.** A 23 minute infusion of hypertonic saline significantly increased serum sodium and plasma osmolality (A&C; $p<0.05$), while there is no change under Time Control conditions (B&D; $p>0.05$).

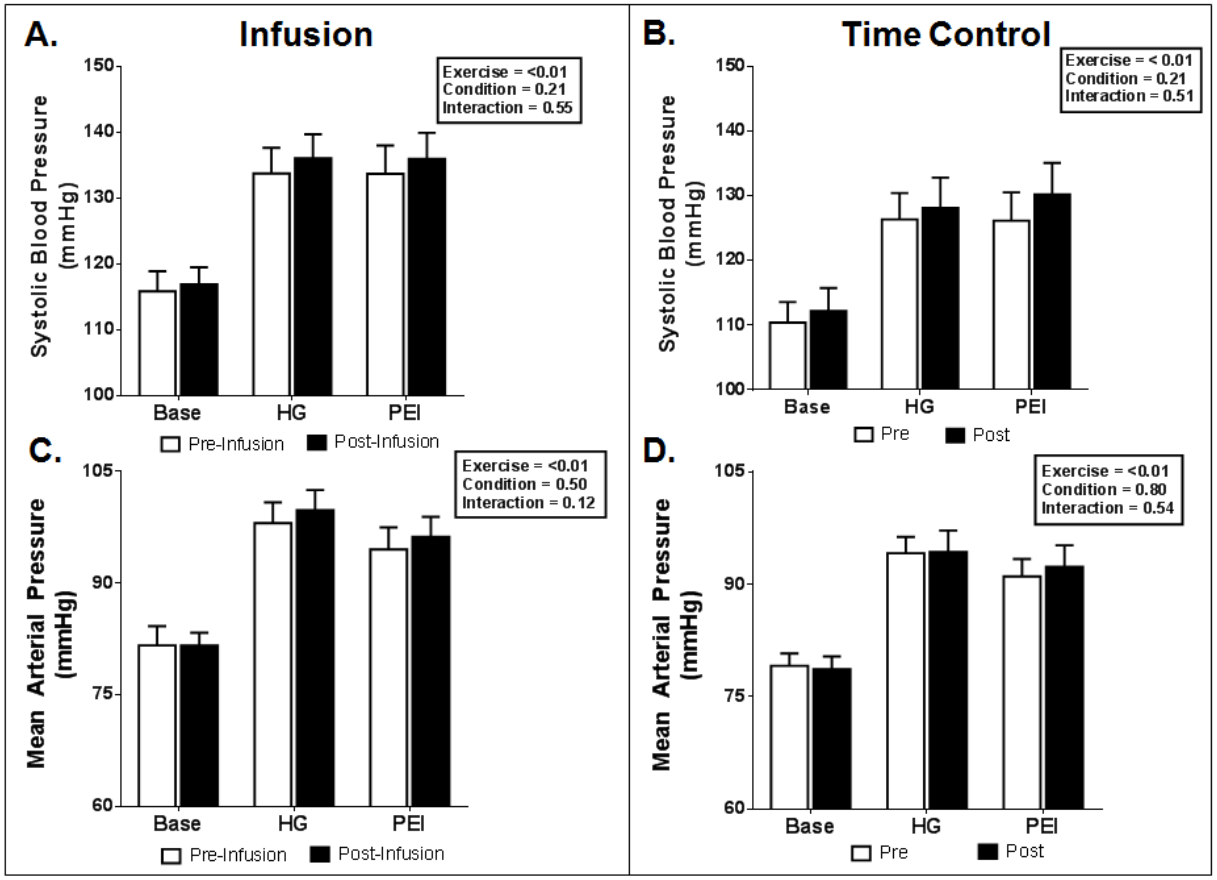

Figure 3.7 **Systolic Blood Pressure and Mean Arterial Blood Pressure Responses under Acute High Sodium and in Time Controls.** Under acute high sodium, BP was not significantly increased compared to the pre-infusion trial (A&C; $p>0.05$). Blood pressure was not significantly increased during the Time Control trial (B&D; $p>0.05$).

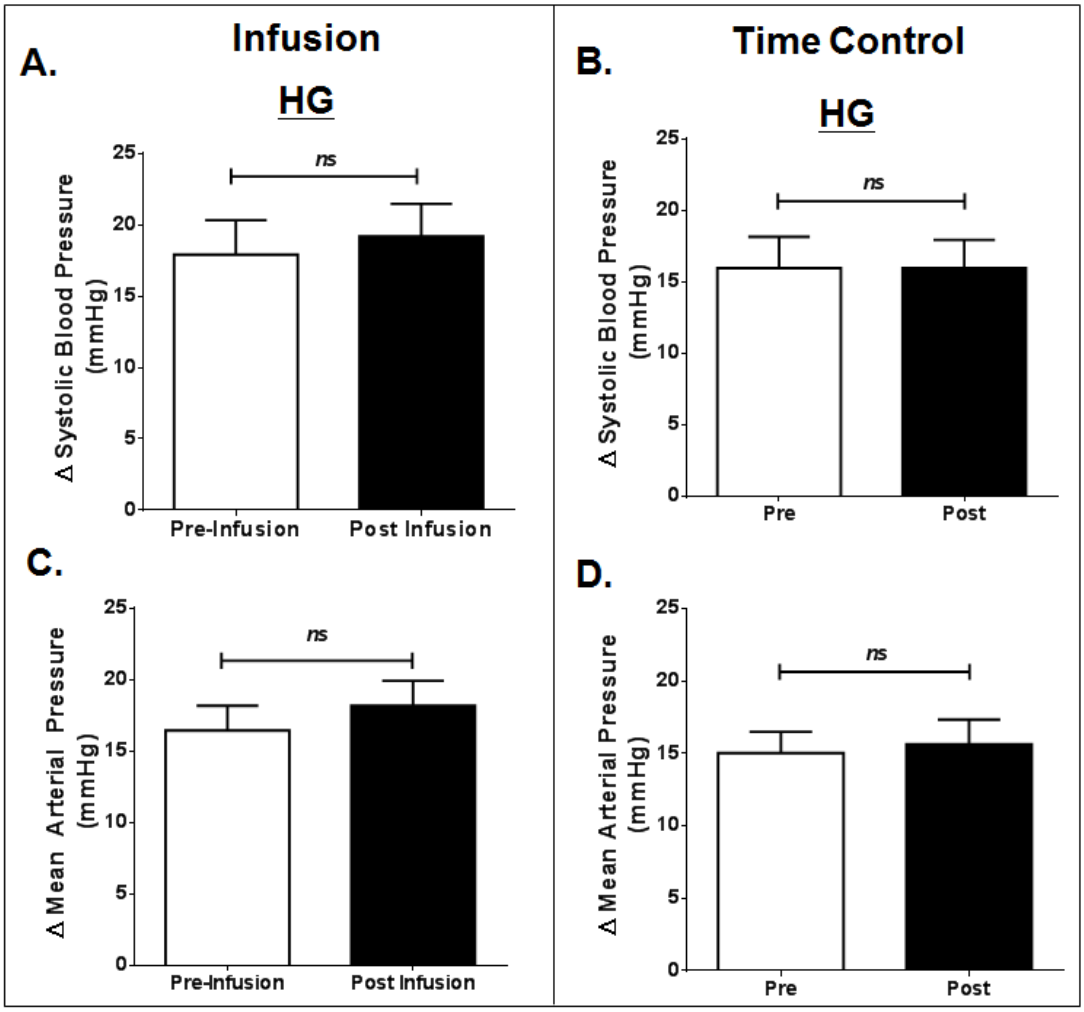

Figure 3.8 **Change in Systolic Blood Pressure and Mean Arterial Pressure Index to Baseline during Pre- and Post Handgrip Trials under Acute High Sodium and in Time Controls.** Acute high sodium does not increase systolic blood pressure or mean arterial pressure during handgrip exercise relative to baseline (A & D; $p>0.05$). There is no change in blood pressure during the Time Control trial relative to baseline (B & D; $p>0.05$).

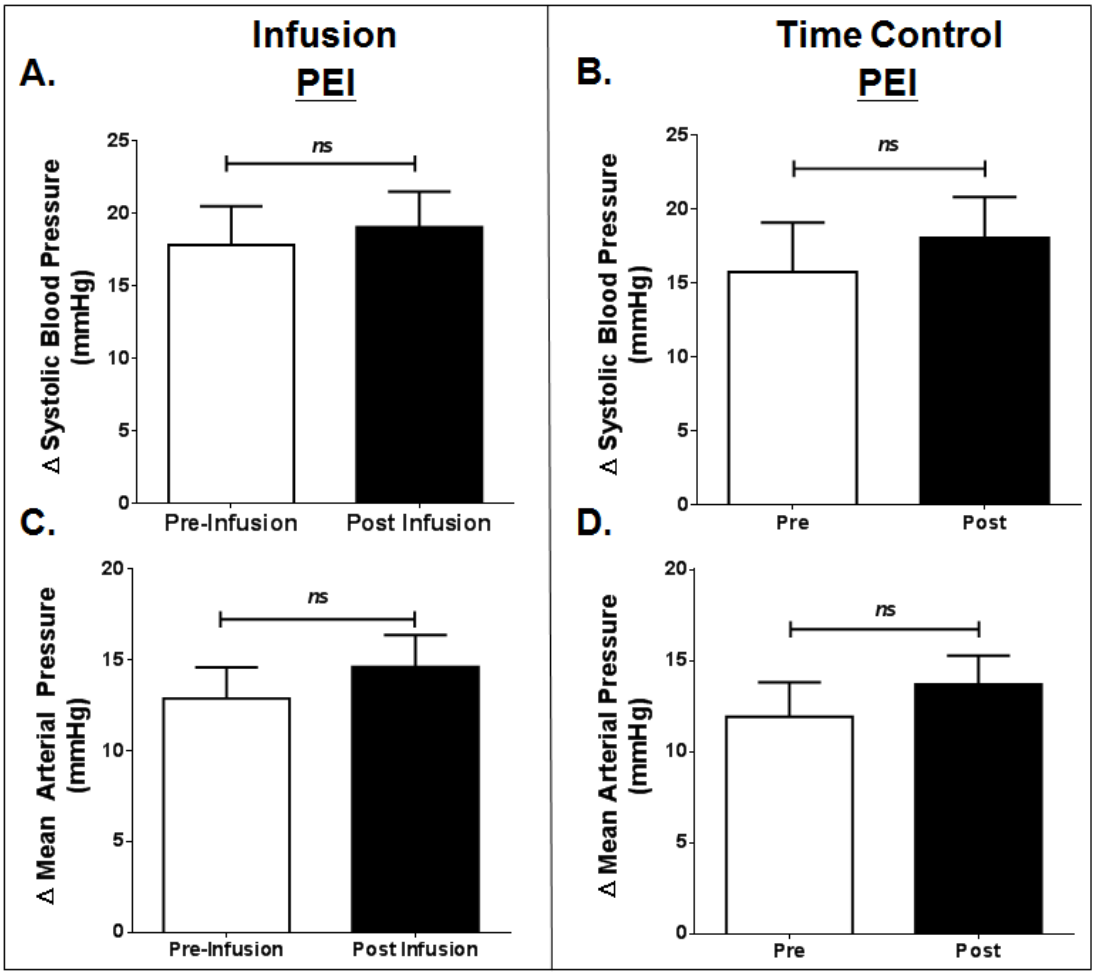

Figure 3.9 **Change in Systolic Blood Pressure and Mean Arterial Pressure Indexed to Baseline during Pre- and Post Exercise Ischemia under Acute High Sodium and in Time Controls.** There was no change in blood pressure or mean arterial pressure relative to baseline during acute high sodium and in Time Controls (A,B,C,D; $p>0.05$).

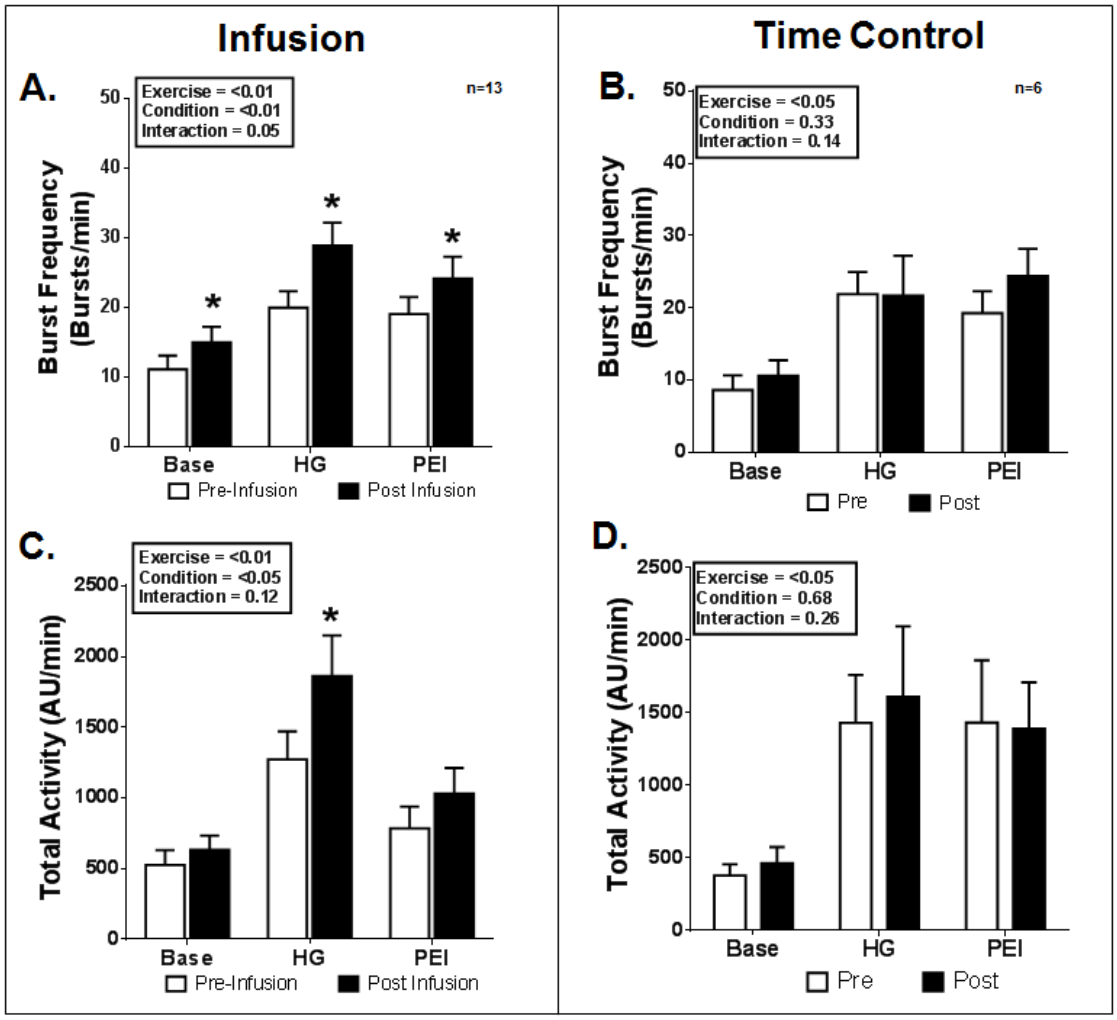

Figure 3.10 **Sympathetic Nerve Activity Responses to Acute High SNa^+ and in Time Controls.** There is a main effect of acute high SNa^+ on measures of sympathetic nerve activity (Panel A & C; main effect of condition $p<0.05$). During the pre to post assessment of sympathetic nerve activity in the Time Control trial, there is no significant increase in sympathetic nerve activity (B & D; main effect of condition $p>0.05$).

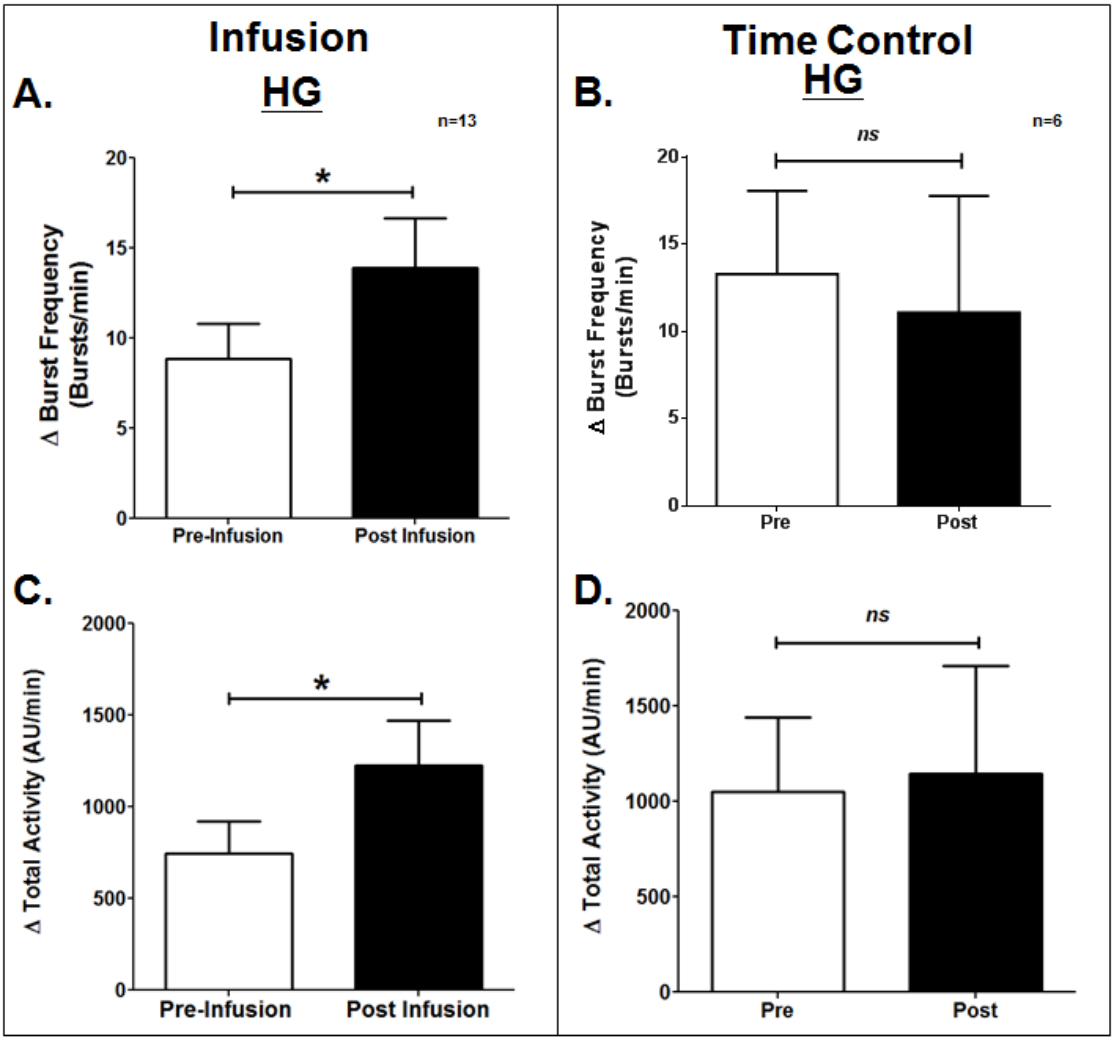

Figure 3.11 **Sympathetic Nerve Activity Responses to Handgrip Exercise are Significantly Increased under Acute High SNa^+.** Sympathetic nerve activity is significantly increased during handgrip exercise under acute high SNa^+ (A & C; $p<0.05$), while there is no significant difference during the Time Control trial during handgrip exercise (B & D; $p>0.05$).

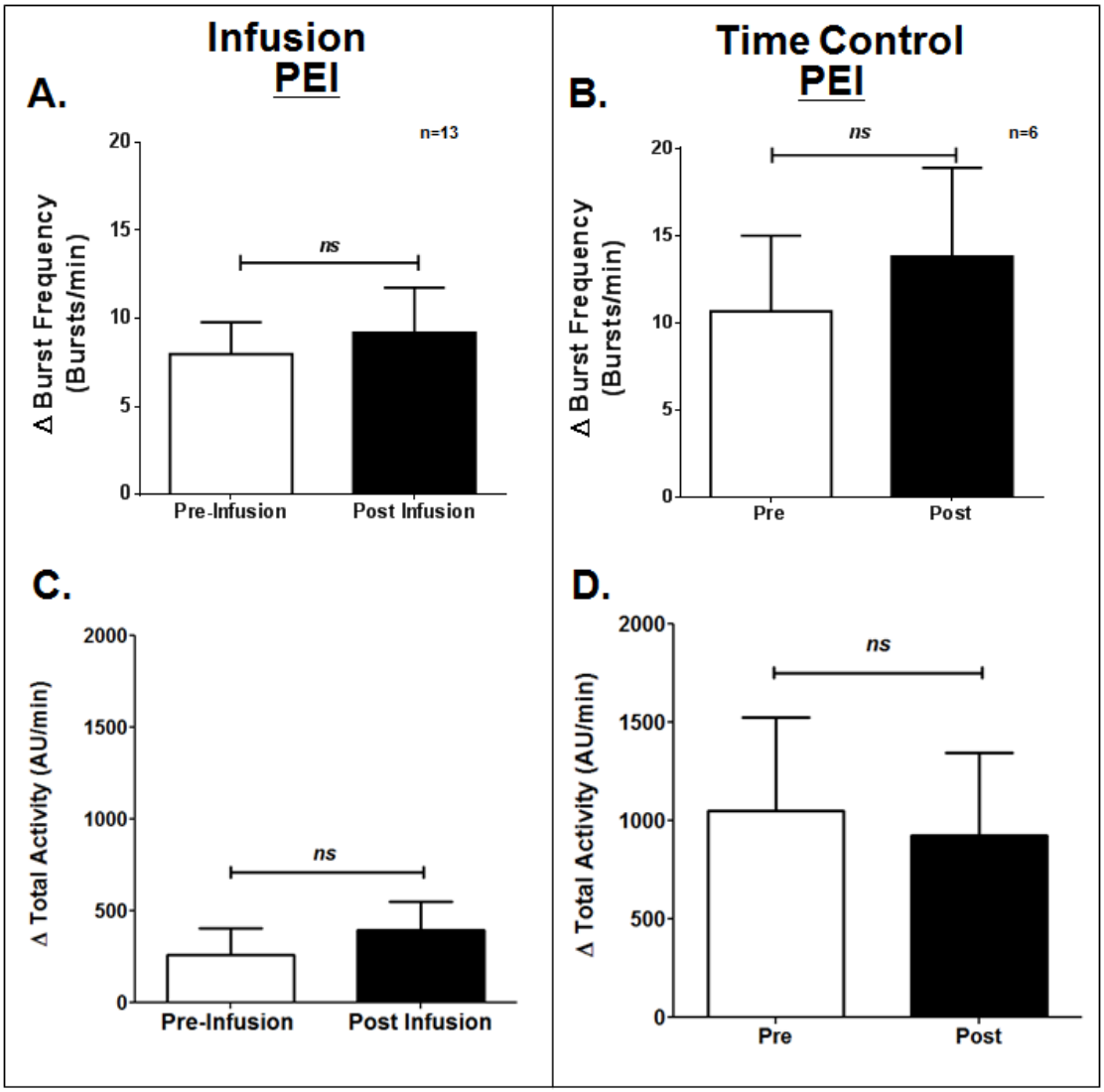

Figure 3.12 **Sympathetic Nerve Activity Responses to Metaboreflex Isolation Remains Unchanged under High SNa^+ and in Time Controls.** Sympathetic nerve activity is significantly increased during handgrip exercise under acute high SNa^+ (A & C; $p<0.05$), while there is no significant difference during the Time Control trial during handgrip exercise (B & D; $p>0.05$).

3.6 Discussion

The two major findings of the study found that among healthy normotensive adults, (1) chronic increases in SNa^+ via high dietary sodium augments systolic BP responses during metaboreflex isolation, and (2) acute increases in SNa^+ had no effect on BP response to handgrip exercise or metaboreflex isolation despite a significant increase in baseline sympathetic nerve activity and sympathetic nerve activity during HG exercise. These are the first findings demonstrating that chronic high SNa^+ increases the BP response to metaboreflex stimulation in normotensive, salt-resistant adults.

In animal studies, AV3V region of the brain has been identified as the primary location sensing increases in SNa^+ and plasma osmolality. Prolonged consumption of HS has been reported to sensitize cardiovascular control centers within the brainstem, which modulate both sympathetic and parasympathetic outflow to the body (1, 2, 44). Further, the sensitization leads to augmented cardiovascular responses to both sympathetic and parasympathetic stimuli. For example, the contraction of hindlimb muscles in normotensive rats consuming HS produced exaggerated BP responses compared to control rats consuming LS and AV3V lesioned rats (114). Further, vagal nerve stimulation caused an exaggerated decrease in BP response in normotensive rats consuming HS compared to rats consuming LS and AV3V lesioned rats (86). Although apparent in rat models, data in humans regarding prolonged HS intake on BP and sympathetic nerve activity is limited. Anderson et al (6) assessed sympathetic nerve recordings in borderline hypertensive and normotensive men. They found that

sympathetic nerve activity was lower after 6 days of HS sodium consumption compared to a LS diet (6). Similar results were found following 8 weeks of sodium depletion (34). However, this might be due to the paradoxical nature to which sodium depletion might also influence the sympathetic nervous system. Several studies have reported that high levels of angiotensin II can stimulate sympathetic outflow and pressor responses (19, 112, 113). Interestingly, the present study did observe an augmented pressor response to sympathetic mediated responses to exercise.

Cardiovascular control during exercise is modulated by several regulatory pathways including central command, the exercise pressor reflex, and the baroreflex (88). During the initial onset of exercise (such as beginning to squeeze a handgrip device), higher brain centers increase sympathetic outflow that cause subsequent increases in BP and heart rate, which is consider as the feedforward control of BP regulation. Both the exercise pressor reflex and baroreflex provide peripheral feedback to the brain. The exercise pressor reflex is comprised of group III and group IV afferent nerve fibers. Group III afferent nerve fibers are considered mechanoreceptive in nature because they primarily respond to stretch and mechanical distortion; while group IV afferent nerve fibers respond to chemical stimulation from exercise such as bradykinin, H^+, histamine, adenosine triphosphate, and lactic acid formation (88). Upon activation, these afferent fibers provide feedback to the brain, which will then increase both sympathetic outflow and BP. Finally, the baroreflex provides beat-to-beat control of BP by inhibiting sympathetic outflow during increased baroreceptor stretch during periods of high BP. During exercise, baroreceptors will reset and maintain higher pressures while mediating sympathetic outflow (88). Both feedforward and feedback pathways are integrated at the RVLM and project down to

target organs controlling BP. Understanding this pathway is important to consider during dietary sodium intake because as discussed previously, increased sodium intake has the potential to alter sympathetic outflow through the sensitization of the RVLM (37). However, the direct mechanism through which dietary sodium alters BP regulation during exercise remains unknown in humans. Consistent findings in rat models suggest dietary sodium affects the central control of BP during chronic sodium intake, however, there are two additional factors that might alter BP regulation during chronic HS leading to an augmented exercise pressor reflex.

The first factor might be due to increased vascular reactivity due to the release of neurotransmitters from sympathetic postganglionic terminals. Sympathetic postganglionic terminals upon activation release neurotransmitters to decrease vascular conductance and increase BP, therefore these terminals might be over-responsive upon activation. However, previous studies in rats found that vascular reactivity remains unaltered during sympathetic stimulation under HS (44, 47, 71, 114). The second additional factor might be due to local afferent sensitization of group III & IV afferent fibers, which are both activated during metaboreflex isolation (17, 60). Three potential sources of local sensitization might be derived from sodium induced inflammation, which might alter bradykinin, histamine, and arachidonic acid concentration. HS has previously been shown to increase inflammation factors such as TNF-α and monocyte chemoattractant protein-1 in healthy normotensive adults (54). However, currently there is no evidence to suggest HS induced inflammation alters the production bradykinin, arachidonic acid, histamine, or that these metabolites further sensitize group III and IV afferents.

Under both chronic and acute conditions, we observed a robust increase in SNa^+ (chronic = 2.6 mmol/L; acute = 3.1). Consistent with previously published data from our laboratory, acute increases in SNa^+ via hypertonic saline infusion augments muscle sympathetic nerve activity, but does not alter BP responses to sympathetic mediated perturbations (36). The current study findings confirm previous findings from Greaney et al (36) that demonstrated acute high SNa^+ increases sympathetic outflow at rest. Interestingly, despite elevated sympathetic nerve activity at rest and during handgrip exercise, BP responses to handgrip exercise remained unaltered in the current study. This might be due to the enhanced baroreflex control of muscle sympathetic nerve activity or reductions in sympathetic transduction. As seen in previous studies, acute increases in SNa^+ increases baroreflex gain, therefore increased gain of the baroreceptors might be buffering excessive rises in BP during handgrip exercise (15, 26). Future studies are needed to experiment whether chronic HS alters baroreflex function in normotensive adults or reduces sympathetic transduction.

3.7 Limitations

There are several limitations to the current study. Despite observing exaggerated systolic BP responses to metaboreflex isolation under chronic sodium, we did not observe significant increases in mean arterial pressure and diastolic BP. As mentioned previously, this might be due to the paradoxical nature through which LS might also alter Ang II response in the body. Future studies are needed to confirm our initial findings. Further, augmented systolic BP responses were found after only 7 days of excess dietary sodium, robust BP responses might take longer to develop as observed in animal studies (86, 114). Future studies are needed to test BP responses to exercise pressor reflex activation following longer exposure to excess dietary sodium. The

measure of salt-sensitivity was utilized to exclude those with salt-sensitive BP during chronic conditions and not during acute conditions. Previous studies have used burdensome methods to assess salt-sensitivity, typically involving large sample sizes, controlled sodium feeding studies, or saline infusions coupled with furosemide (diuretic) administration following sodium/volume depletion (95, 106). Although we were unable to address salt-sensitivity, the acute hypertonic saline infusion did not increase BP in our cohort of healthy normotensive adults. Therefore, it would be inappropriate to arbitrarily classify participants as salt-sensitive utilizing the methods of the current study. Finally, the current study only measured BP responses to 40% isometric handgrip exercise and cannot rule out a potential ceiling effect for BP responses at the provided work intensity. Future studies should utilize a variety of exercise intensities (20%, 30%, etc) and modes (static vs. rhythmic), and other sympathetic mediated stimuli (cold pressor test or venous distension) to determine the full effect of chronically elevated SNa^+ on BP responses.

3.8 Conclusions

In conclusion, chronic but not acute increases in SNa^+ augment systolic BP response to metaboreflex isolation in normotensive salt-resistant adults. While acute increases in SNa^+ increases muscle sympathetic outflow, BP responses to increased sympathetic outflow during handgrip exercise remained unaffected. Our findings provide the first evidence in humans, that chronic HS intake alters the metaboreflex in salt-resistant normotensive adults potentially through elevated SNa^+. Further, our findings might have clinical relevance in that consumption of HS leading to exaggerated BP responses might predispose individuals for future hypertension and

cardiovascular disease, even in a population not to be considered at-risk of cardiovascular disease (59).

Chapter 4

DISSERTATION CONCLUSIONS

4.1 Summary

Dietary sodium has been shown to increase cardiovascular disease risk in individuals with salt-sensitivity, hypertension, kidney disease, and hyperaldosteronism (4, 8, 22, 58, 94, 99, 100). Importantly, recent evidence in rats suggests that chronic SNa^+ might alter BP regulation through changes in neural plasticity (92). However, limited data in humans has addressed whether or not SNa^+ alters BP regulation. Therefore, the following aims of the current dissertation were designed to examine whether or not chronic and acute increases in SNa^+ increases systolic BP variability and BP responses to exercise pressor reflex activation.

The first aim focused on assessing BP variability during chronic and acute increases in SNa^+. Chronic high SNa^+ was accomplished by having normotensive, salt-resistant participants consume a controlled 7 days of HS (300 mmol/day) and LS (20 mmol/day) diets in random order. Systolic BP variability was assessed by participants wearing a 24h BP monitor on the final day of each sodium diet. Participants were split based on a 2 mmol change in SNa^+ (Δ SNa^+= HS-LS) to match the expected change during the acute condition. Acute high SNa^+ was accomplished by infusing 3% NaCl for 23 minutes; a time control was also performed as a sodium neutral condition and to account for time. The results found that chronic increases in SNa+ increased systolic BP variability in only those that had an increase in SNa^+.

However, acute increases in SNa^+ did not increase BP variability. The current findings are the first of its kind to find that normotensive, salt-resistant adults have increased BP variability during increases in SNa^+. This information further suggests that sodium sensing regions may influence 24h BP variability during HS. Importantly, increased BP variability independent of mean BP has been shown to increase cardiovascular disease risk and target organ damage (84, 97, 103).

The second aim focused on assessing whether chronic and acute increases in SNa^+ increased BP responses to exercise pressor reflex activation. Chronic increases in SNa^+ were performed using the same controlled feeding study as previously mentioned. On the final day of each diet, BP responses to handgrip exercise and metaboreflex isolation was measured. Acute increases in SNa was accomplished by infusing 3% NaCl as previously mentioned, BP and muscle sympathetic nerve activity responses to handgrip exercise and metaboreflex isolation were measured pre and post NaCl infusion. The results found that chronic increases in SNa^+ significantly increased systolic BP responses to metaboreflex stimulation, and acute increases in SNa^+ increased resting sympathetic nerve activity and sympathetic nerve activity during handgrip exercise. These findings provide the first evidence in humans that chronically elevated SNa^+ increases BP response the metaboreflex isolation in normotensive salt-resistant adults. Studies in rats have previously shown that 14 to 15 days of HS consumption leads to exaggerated BP responses to exercise pressor reflex simulation (114). Therefore, the findings of the present study provide a translational link between rodent and human studies.

4.2 Perspectives

Normotensive salt-resistant adults make up the majority of young and middle-aged adults in the United States (105), and this group regularly consumes excess dietary sodium. However, recent evidence suggests that excess dietary sodium has been demonstrated to adversely affect vascular function in normotensive salt-resistant adults (20, 21, 23, 35, 58). Studies in rats have also suggested that SNa^+ might be responsible for increased BP variability and exaggerated exercise pressor reflex responses through sodium sensing mechanisms in the brain (86, 114). The findings of the current dissertation provide evidence that chronic increases in SNa^+ increases systolic BP variability and augments systolic BP responses to metaboreflex isolation. However, acutely increasing SNa^+ leads to greater sympathetic nerve activity at rest and during handgrip exercise, but does not alter BP responses during exercise or metaboreflex isolation.

To date, limited studies have assessed BP responses to exercise during a HS diet, and no studies have examined the affect SNa^+ on BP variability and exercise pressor reflex activation. The novel findings of the current dissertation provide new insight on the chronic and acute effects of increasing serum SNa^+. Future studies are needed to confirm these findings during longer exposure to elevated SNa^+.

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Appendix A

IRB HUMAN RESEARCH APPROVAL FORM