BLOOD PRESSURE REACTIVITY AND SALT SENSITIVITY IN NON-HYPERTENSIVE ADULTS

by

Evan L. Matthews

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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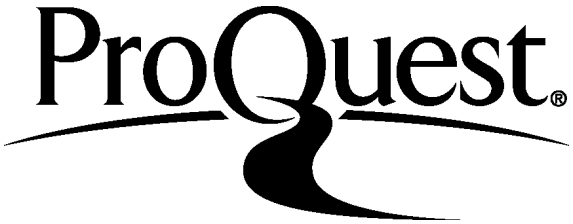

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Approved: _____

William B. Farquhar, Ph.D.
Chair of the Department of Kinesiology & Applied Physiology

Approved: _____

Kathleen S. Matt, Ph.D.
Dean of the College of College of Health Sciences

Approved: _____

James G. Richards, Ph.D.
Vice Provost for Graduate and Professional Education

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

William B. Farquhar, Ph.D.
Professor in charge of dissertation

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Megan M. Wenner, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

David G. Edwards, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Sean D. Stocker, Ph.D.
Member of dissertation committee

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LIST OF ABBREVIATIONS

AU, arbitrary units

B, black

BMI, body mass index

BP, blood pressure

Burst In, burst incidence

CFA, common femoral artery

CVLM, caudal ventrolateral medulla

DBP, diastolic blood pressure

DSR, Dahl salt resistant

DSS, Dahl salt sensitive

F, female

HR, heart rate

HS, high dietary sodium

IRB, Institutional Review Board

IV, intravenous catheter

LS, low dietary sodium

M, male

MAP, mean arterial pressure

MSNA, muscle sympathetic nervous activity

MVC, maximum voluntary contraction

Na^+, sodium

NE, norepinephrine

NO, nitric oxide

NTS, nucleus tractus solitaries

O, other

OsM, osmolality

OVLT, organum vasculosum of the lamina terminalis

PEI, post exercise ischemia

R Id, resistance index

RPE, ratings of perceived exertion

RVLM, rostral ventrolateral medulla

SBP, systolic blood pressure

SD, standard deviation

SFO, subfornical organ

SS, salt sensitivity

TAmean, time averaged mean

W, white

ABSTRACT

The central goal of this project was to determine the relationship, if any, salt sensitivity (SS) status has with the autonomic nervous system control of blood pressure (BP) during rest and sympathoexcitation. This is important because SS is thought to be linked to the autonomic nervous system, yet very little human based research has been done on the topic. Additionally, individuals with high degrees of SS are more likely to develop hypertension and die of cardiovascular disease. We enrolled 50 non-hypertensive adults predetermined for SS status to undergo testing during rest, isometric handgrip exercise, post-exercise ischemia (PEI), venous distension, and a cold pressor test. We measured continuous BP, muscle sympathetic nervous activity (MSNA), and limb (femoral artery) blood flow. Additionally, sympathetic baroreflex sensitivity was assessed during rest and PEI. BP reactivity was not related to SS status during any of the sympathoexcitatory tests (all $p>0.05$). Furthermore, no relationship existed between SS status and the response of MSNA or limb resistance during PEI, venous distension, or the cold pressor test (all $p>0.05$). However, resting MSNA (burst frequency $r=0.47$, $p=0.041$; and burst incidence $r=0.46$, $p=0.046$) was directly related to SS status. During handgrip MSNA response was inversely related for all measures (all $r<-0.50$; $p<0.05$) except burst incidence ($r=-0.37$; $p=0.129$), as was limb resistance ($r=-0.38$; $p=0.016$). Conversely limb blood flow was positively related to SS status ($r=0.33$; $p=0.036$). This suggests that although the BP response was not related to SS status during handgrip, those with the greatest SS support the BP response with increased blood flow rather than increased sympathetic activity and resistance. Additionally, sympathetic baroreflex sensitivity

did not relate SS status at rest or during PEI. Our findings suggest that resting sympathetic activity, but not sympathetic reactivity or sympathetic baroreflex sensitivity are related to salt sensitivity status in non-hypertensive adults. More research is needed to determine the cause and potential effects of these findings.

Chapter 1

REVIEW OF LITERATURE

1.1 Introduction and Overview

Salt sensitivity (SS) is a measure of how much blood pressure (BP) increases following an increase in dietary sodium. SS follows a normal distribution and is often divided to form groups for ease of analysis. Many clinically important populations display exaggerated SS including individuals with hypertension (132). Having high levels of SS has been linked to increases in cardiovascular disease risk (84).

SS among normotensive adults is lower than that of hypertensives, but still displays heterogeneity. When normotensives are split at the median into higher and lower SS groups, individuals within the higher SS group are more likely to become hypertensive (8). Children with high levels of SS also display greater development of hypertension (86). The relationship of high SS and hypertension risk is especially important as hypertension is a significant risk factor for cardiovascular related death (85), which may explain the relationship between highly SS adults and poor survival rates during follow-up (130).

The cause of the heterogeneous response to changes in dietary sodium is likely multifactorial (69), but emerging evidence from animal literature suggests that the central nervous system is integral to this response (118). Differences in SS may be mirrored by BP responsiveness to peripheral reflex stimulation (64). This may be due to a shared central mechanism between the phenomenon's of high SS and high peripheral reflex stimulation responsiveness. Additionally, abnormalities with the

arterial baroreflex may exist in those with elevated SS. Exaggerated cardiovascular reactivity to the demands of daily life may result in greater BP variability. This would contribute to the higher mortality in those with high SS. The following review of available literature will further explore the topics mentioned thus far.

1.2 Concept of Salt Sensitivity

Consistency is lacking, even among large epidemiological studies (58, 119), for whether dietary sodium induces hypertension. In fact, the effect of dietary sodium on BP is highly variable. Over half a century ago, Dahl et al (25) sought to determine if this variability within BP responsiveness to dietary sodium is the result of genetically driven differences between participants, or simply variability within the measurement of BP. To test this, they fed rats a high sodium diet and paired them for breeding for three generations according to their BP response; high responders were bred with high responders, and low responders were bred with low responders. Through this they were able to create rats whose BP responsiveness to dietary sodium matched that of their lineage. This confirmed that genetics plays a role in the heterogeneity of the BP response to dietary sodium (i.e. SS) (25). More recently, evidence in humans has confirmed heritability of SS status (45). Now many gene variations are associated with the various mechanisms thought to cause elevated SS (e.g. renin-aldosterone-angiotensin system, sympathetic nervous system, endothelial nitric oxide synthase, etc.), of which recent reviews are available (30, 111). It is this measurement of the individual SS of BP that has been the subject of numerous studies.

1.3 Experimental Designs for the Assessment of Salt Sensitivity

Studies probing the concept of SS within humans have varied widely in the dietary protocol, the criterion for division of individuals into groups, and the terminology used to describe these groups.

The assessment of SS normally requires participants to consume a two phased dietary perturbation. Ideally, the phases of the dietary perturbation differ solely by the sodium content of the food consumed. The measurement of SS is calculated by examining the within participant change in BP from the lower to higher sodium diets, LS and HS respectively. Thus, an individual with a much higher BP on HS compared to LS would score a high value for SS; conversely an individual with roughly equivalent blood pressures between sodium phases would be evaluated to have a low level of SS. The sodium contents of these phases, the absolute difference of phase-to-phase sodium content, and lengths of these phases are not uniform between studies; the following is a list of examples: units mmol Na+/day: HS 200x14d vs. LS 20x9d (14), HS 300x14d vs. LS 50-100x14d (68), HS habitual intake + 170x14d vs. LS habitual intake (33), HS 300x7d vs. LS 10x7d (16). Additionally, an abbreviated two day protocol using saline infusions on day one (i.e. HS) followed by dietary induced LS with diuretic administration have been favored by some for their brevity (132). It is worth noting that protocols based solely on controlled feeding are considered to be the gold standard due to their more direct applicability to the topic of dietary sodium. The most reliable (i.e. most reproducible) SS assessments control participant sodium consumption prior to providing high or low sodium diets (111), presumably due to the time required to achieve sodium balance.

Human studies on the topic of SS typically use criterion to divide participants into two or more groups according to their individual response to the SS assessment.

This is not done because of a dichotomous response to the dietary sodium perturbation. Indeed, this response is unimodal and normally distributed (see figure 1.1) (51), and boundaries are only defined to allow for direct comparison of individuals separated into groups. Often the boundary criterion is an arbitrary cut point without direct rational offered. This can result in unequal group sizes, potentially posing statistical problems of inadequate power and unequal group variances. If the goal of a study is to examine within group effects of sodium in participants without a large increase in blood pressure, choosing an a priori arbitrary exclusion cut point can be helpful as it allows for a predictable group mean BP response to be near 0 mmHg (i.e. group salt resistance). Likewise, if the goal is a direct comparison of new data to the data of that of a previous study, it is helpful to select the same arbitrary cut point as the study of interest. Conversely, if the goal of the study is to compare results to the general body of literature on SS, choosing which arbitrary cut point to follow is extremely difficult given the inconsistency within the literature; list of examples: 3 mmHgΔ (116); 5 mmHgΔ (113); 7 mmHgΔ (16); 10 mmHgΔ (79); 5%Δ (33); 10%Δ (84). There are also inconsistencies in the calculation of the SS response, type of BP measurement used for calculations, and method for BP measurements; lists of examples: *calculation of SS response* - ΔBP (14), %ΔBP (45), salt sensitivity index = ΔBP/ΔNa+ excretion x 1000 (19), *type of BP measurement used for calculations* - systolic BP (83), diastolic BP (109), mean BP (16), *and how BP measurements were obtained* - office BP (132), 24hr ambulatory BP (114). If the goal is to compare between groups based on SS status, careful consideration of the literature can lead to some suggestions. Of note, SS increases as baseline BP increases (51), therefore studies where baseline BP may compound results a calculation of SS

made relative to BP (i.e. %Δ) should be considered. Conversely, if the BP spread is similar between comparison groups and/or conditions results will likely be similar between relative and absolute measures of SS (45). Also, rather than dichotomizing participants based on arbitrarily chosen cut points, viewing SS as a continuous variable with degrees of sensitivity appears to be a more sensible approach (71). With these considerations in mind, viewing SS as a normally distributed unimodal variable (see figure 1.1) requires examining SS without division into groups (46) or with statistically meaningful divisions; list of examples: median split (8), tertiles (97), quartiles (19)).

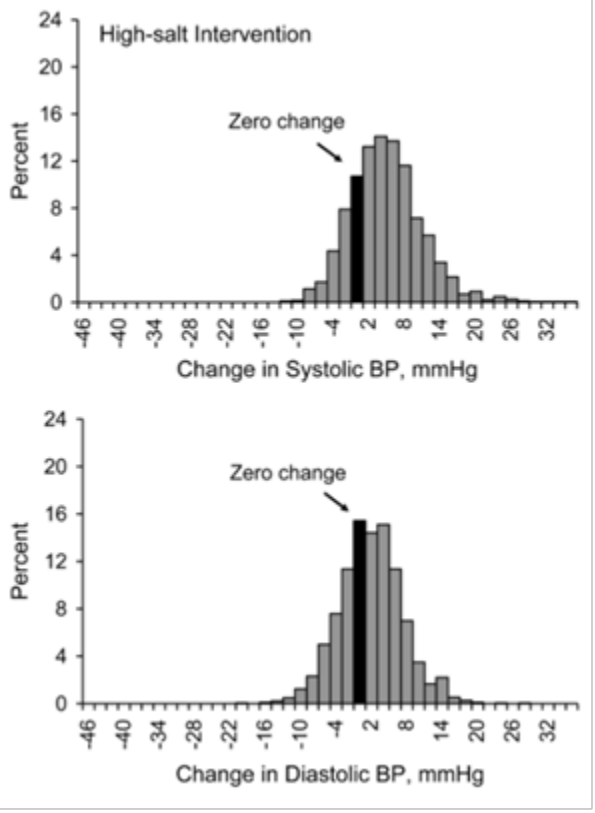

Figure 1.1: **Depiction of salt sensitivity distribution**. Percent of total participants (n = 1,906) by change in blood pressure, mmHg, on a high salt diet (307.8 mmol Na+/day x 7 days) vs. low salt diet (51.3 mmol Na+/day x 7 days). Adapted from He et al. 2009.

When the SS continuum is separated into groups, the names assigned to these groups vary from study to study. Participants grouped due to a large rise in BP with increases in dietary sodium are termed salt sensitive (14) or sodium sensitive (10)), while participants grouped due to little to no rise or a decrease in BP are termed salt resistant (14), non-sensitive (84), non-salt sensitive (63), or sodium insensitive (10). Of these terms the most commonly used are salt sensitive and salt resistant. Some researchers choose to create a small buffer zone of individuals that will be excluded

from analysis in an attempt to widen the difference between groups; this third group is termed indeterminate (132). Additionally, the "salt resistant" individuals displaying higher BP on the LS vs. HS are sometimes further defined using other terminology (e.g. counter-regulatory (133); inverse salt sensitive (34)).

The additional classification of a subset of salt resistant individuals into a counter-regulatory group is rare and may be unnecessary as it is not likely the result of a unique mechanism. The abrupt onset of the LS phase does cause a large elevation in the renin-angiotensin-aldosterone system (RAAS) and circulating norepinephrine (NE) (42), that conceivably may cause an overshooting affect leading to elevated BP during the LS phase in some individuals. If overshooting were a significant factor, sodium loading studies without an abrupt drop in dietary sodium during the LS phase (e.g. when habitual intake is used in place of a LS phase), would not result in a substantial number of individuals experiencing an inverse BP response. Yet research fitting this description continues to result in counter-regulating participants (33). Conversely, the abrupt onset of the HS phase could conceivably cause over-suppression of RAAS and circulating NE, which would lead to a paradoxical drop in blood pressure. However, sodium restriction studies where habitual intake serves as the HS phase also results in large numbers of counter-regulating participants (132). Additionally, studies that categorize participants into multiple groups based on the distribution of responses (e.g. quartiles) cause the lowest SS group to be made solely of counter-regulators, yet a step wise linear relationship exists between groups and the study variable of interest (19). This suggests inverse responders are not a unique group, but part of the SS continuum. Therefore considering inverse responders as a unique subset is likely unnecessary as the relative decrease in BP during the HS phase is probably the result of individuals

with a high degree of intra-individual BP variability masking a very low degree of SS. Evidence to support this hypothesis is displayed in figure 1.2 (74, 134). Luft et al. (74) found that with step-wise increases in sodium levels, all participants eventually have a positive %ΔBP (see figure 1.2 A). Furthermore, the high responders and low responders are still high and low responders, respectively, at the highest sodium levels. This is despite the fact that sodium intake levels well beyond 300mmol/d are rare in daily life. Evidence of this is displayed in figure 1.2 B; a strong relationship exists between SS classified based on a high sodium phase of 300mmol/d and SS classified with much higher high sodium phases, 1200 or 1500mmol/d. This suggests that SS can be accurately assessed with sodium levels much higher than what is common in daily life or most SS research designs, and the number of counter-regulators decreases as the level of sodium consumed during the high sodium phase increases.

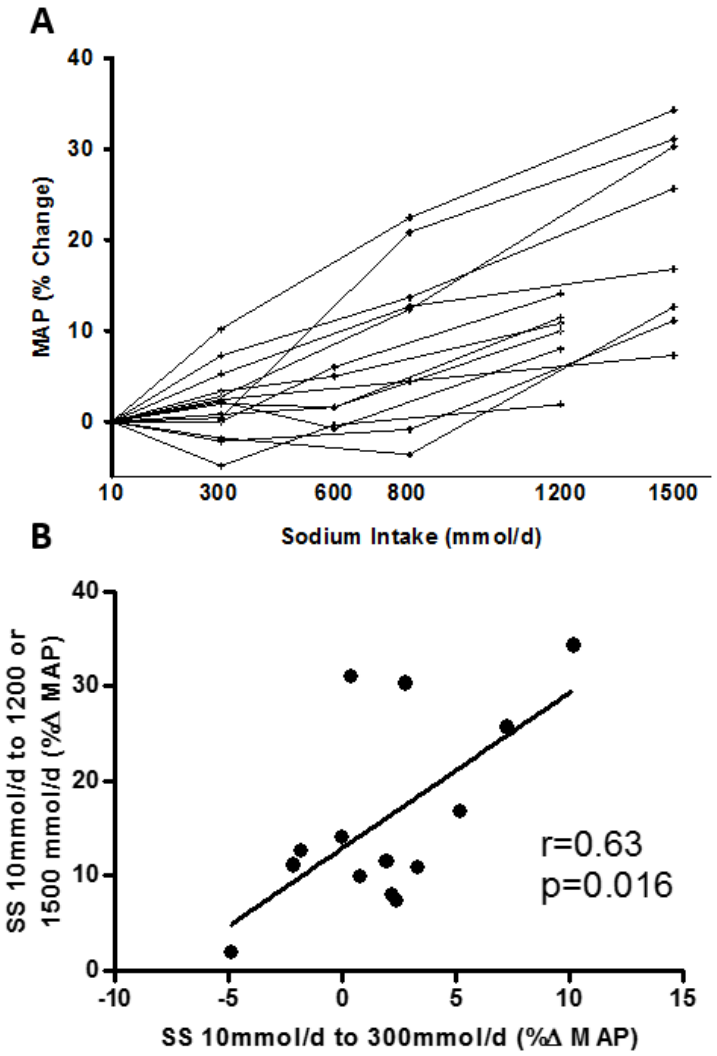

Figure 1.2: **Depiction of Changes in Mean Arterial Pressure with Varying Levels of Sodium Intake.** A. Increases in sodium intake eventually cause all individuals to have a positive percent change in mean arterial pressure. Figure adapted from Weinberger (133). B. Salt sensitivity for sodium intake at 300mmol/d vs. highest sodium intake, 1200 or 1500mmol/d, are highly correlated. Data adapted from Luft et al. (74).

Given the information reviewed thus far, it seems reasonable to suggest viewing SS as a continuum. With this in mind, the utilization of regression based statistics seems appropriate. However, it is sometimes beneficial to lump individuals based on cut points as this is beneficial to conceptualize findings, or determine treatments for conditions with normal distributions (e.g. classification of resting blood pressure). With this in mind, the measurement variable is "salt sensitivity" which is nearly indistinguishable from the term "salt sensitive" which is often used to describe the high responding group. Hence, if SS is viewed as a grouped continuous variable, the groups are sometimes referred to according to the chosen statistical dividing strategy; list of examples: tertile: 1st, 2nd, 3rd (97) or low SS, moderate SS, high SS (7, 37); quartile: 1st, 2nd, 3rd, 4th (19)). If the chosen division is a median split then there are only two groups with either higher SS or lower SS (8). Investigating SS using statistical groupings removes subjectivity and arbitrarity of group boundaries. However, consideration to viewing the group as a whole still merits consideration given the normal distribution associated with SS.

1.4 Salt Sensitivity within Special Populations

Even with the lack of uniformity in assessing SS, many meaningful observations regarding SS have been established that are sufficiently robust to overwhelm differences in methodology. Some of the earliest observations found higher SS among African Americans, aged adults, hypertensives (132) and women (51, 67). This is evident when plotting SS histograms between groups as a rightward shift in the distribution for groups with higher SS (see figure 1.3). The claim that women are more salt sensitive than men has received some resistance because as

Weinberger (133) points out, women typically have lower body mass than men leading to a greater relative sodium stimulus that is unaccounted for by study designs feeding all participants the same sodium load. However, this does not mean that SS assessment does not provide information especially relevant to women. A recent study found that normotensive premenopausal women with a history of severe preeclampsia have higher SS than matched counterparts who had normal BP throughout pregnancy (81). This finding suggests that a lack of ovarian hormones, similar to that occurring during menopause, allows for the expression of hypertension in women with high SS. Therefore, SS assessments may provide clinical insights into future outcomes of premenopausal women. SS status also provides clinical insight in adults with hypertension, in that high degrees of SS are associated with greater rates of cardiovascular events on follow-up (84).

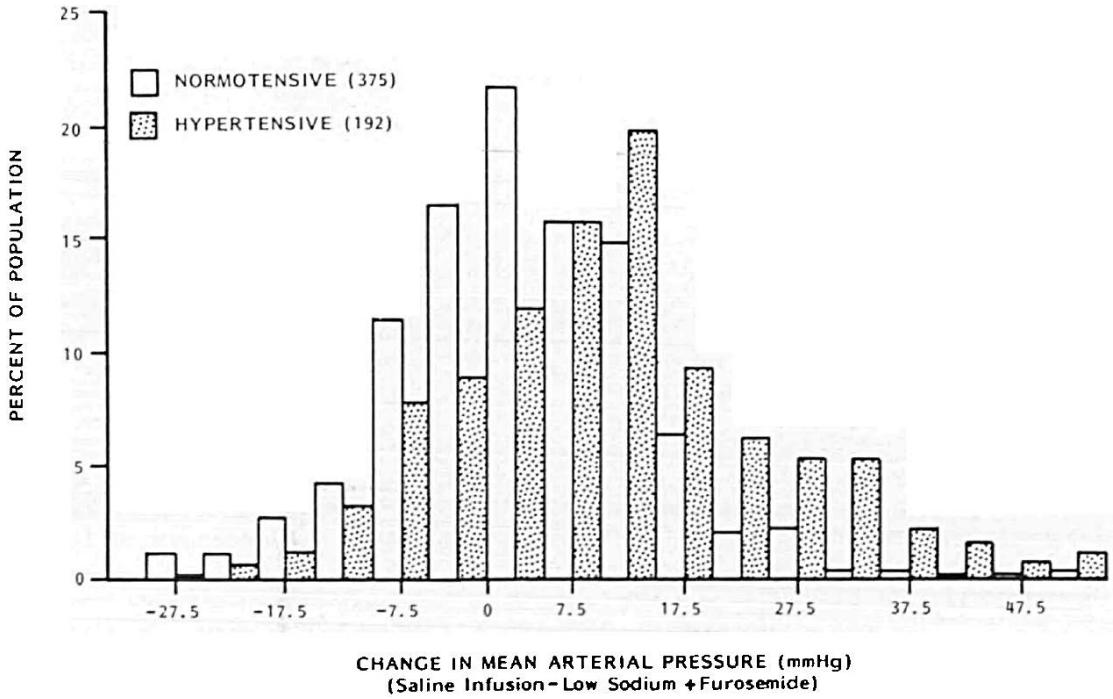

Figure 1.3 **Salt Sensitivity Distribution is Shifted to the Right in Hypertensive Adults.** Weinberger et al. (132) performed an acute SS tests on normotensive and hypertensive adults and found greater SS to be accompanied by a rightward shift in the entire distribution.

1.5 Salt Sensitivity within Apparently Healthy Participants

Although individuals without hypertension typically display lower levels of SS compared to those with hypertension, there is still heterogeneity in the BP response to varied dietary sodium (132) that provides clinical insights into future outcomes. Weinberger and Fineberg (131) found that normotensive adults with high levels of SS had a greater increase in systolic BP and diastolic BP over time than those with lesser degrees of SS (131). In one study with healthy normotensive adults, those in the upper 50% for SS, had 37.5% higher prevalence of hypertension after a 15 year follow up period than the lower 50% for SS (8). Another study found similar results in adults using different methods for SS classification (120). SS as a predictor of future hypertension has been recently confirmed in a study of children, ages of 6-15 years and followed for 18 years, suggesting this relationship exists even in the earliest years of life (86). The relationship between SS status and the development of hypertension is clinically meaningful as hypertension is a major contributor to cardiovascular disease, which is the leading cause of death in the United States (85).

Evidence exists showing that both animals and humans who display high degrees of SS have poorer survival rates than their less SS counterparts. While developing the animal model of genetic SS, Dahl et al. (25) fed Sprague-Dawley rats high sodium chow and selected the rodents who were “most sensitive” to the sodium protocol for the Dahl-Salt-Sensitive (DSS) lineage and the “less sensitive” rats for the Dahl-Salt-Resistant (DSR) lineage. Following the first cycle of selective inbreeding, an unfortunate and near devastating pneumonia epidemic swept the study animals causing 45% of the rats bred for high levels of SS to die, while only 10% of the

animals bred for lower SS died (55 DSS with 25 deaths; 58 DSR with 6 deaths). This provided the first evidence of increased mortality with higher levels of SS. Subsequently, a large longitudinal study done by Weinberger et al. (130) found that normotensive individuals with a high degree of SS have poorer survival rates, similar to hypertensive participants. Importantly, of those who died of cardiovascular disease, significantly more fell within the studies higher SS group.

1.6 Causes of Salt Sensitivity

Due to the worsened mortality (130) and cardiovascular outcomes (84, 130) associated with high magnitudes of SS, there is a strong interest within the scientific and clinical communities to understand SS and its potential causes. Unfortunately, a singular cause of the differential phenotypic BP response to dietary sodium is unknown. The mechanism explaining why some individuals have high degrees of SS is now believed to be multifactorial as physiological systems intertwine allowing for renal, hormonal, vascular, and neural mechanisms (69). It is likely that the root dysfunction can happen within any of these key systems, and thus the cause of high SS may be different between people. An extensive list of potential mechanisms is available in a recent review article by Kotchen et al. (69) of which a thorough exploration is beyond the scope of this literature review. Regardless there are a select number of viewpoints/mechanisms worth discussing here: impaired renal natriuresis, decreased bioavailability of nitric oxide, and central nervous system over reactivity.

1.6.1 Salt Sensitivity and Renal Natriuresis

The Guyton model of SS, the oldest and most well-known model, associates impaired pressure natriuresis within the kidney with the eventual rise in BP during

high dietary sodium. This impairment in natriuresis may have multiple mechanisms such as loss of nephrons, increased distal and collecting tubule reabsorption, or decreased glomerular filtration coefficient (48). The previously listed mechanisms may be brought on by intrinsic abnormalities, damage to the kidney, or the consequence of abnormal input from other physiological systems (e.g. imbalanced hormonal secretion of aldosterone or over stimulation of the kidney arterioles by the sympathetic nervous system). Regardless of the root cause, in this model, blood volume increases resulting in whole body autoregulation of feed arterioles in an attempt to prevent over perfusion of bodily tissues. This large scale autoregulation results in increased total peripheral resistance and BP. Conversely, an individual with a lower degree of SS would experience a more proportionate rise in natriuresis with elevated sodium intake preventing a dramatic increase in BP (47).

1.6.2 Salt Sensitivity, Vasculature, and Nitric Oxide

It is possible that the dysfunction in highly SS individuals starts within the blood vessels themselves. The concept of "vascular" SS has been introduced relatively recently (94). The surface of the endothelium is lined with negatively charged biopolymers called glycocalyx that have the ability to prevent sodium from reaching the membrane of the cell (70). This lining becomes damaged over time with high sodium exposure allowing sodium to reach the epithelial sodium channels and gain access into the endothelial cells (93). When endothelial cells are exposed to elevated sodium levels they stiffen and produce less nitric oxide (NO) when exposed to shear stress, i.e. endothelial dysfunction (94). As a major molecular signal for vascular relaxation, a body wide lack of NO would likely cause an increase in total peripheral resistance and BP. Therefore, if the development or preservation of

endothelial glycocalyx is different between individuals it would result in a heterogeneous effect of dietary sodium on BP. In addition to sodium's potential to stiffen endothelial cells, NO levels can be lowered by sodium due to an imbalance between antioxidants and oxidants (72, 91, 92, 136). Regardless of the cause of lowered NO, this can result in elevated SS. As an extreme example, mice with endothelial nitric oxide synthase knockout experience high levels of SS and greater increases in BP during activity (73).

1.6.3 Sodium, Salt Sensitive Hypertension, and Central Nervous System Reactivity

An ever increasing amount of evidence points towards the nervous system as a potential mechanism for SS of BP. We now know that sodium does have a direct impact on the central nervous system affecting blood pressure control. It is likely an important component of salt sensitive hypertension, and potentially involved in determining SS of normotensives. The next few paragraphs will discuss these claims in detail.

1.6.3.1 The Effect of Sodium on the Central Nervous System in Normotensive Salt Resistant Rats

Although most of the brain is thought to be defended from alterations in blood sodium levels by the blood brain barrier, sodium does impact central nervous system function. The anteroventral third ventricle (AV3V) of the brain includes the subfornical organ and organum vasculosum of the lamina terminalis (OVLT) which lack complete blood brain barriers, and are capable of sensing blood sodium levels. The importance of AV3V brain region for the interaction between sodium and BP was assessed through a set of experiments on the Sprague-Dawley rat strain (3). This

study employed selective brain lesioning and direct chemical stimulation (L-glutamate) during HS and found a key control center of the sympathetic nervous system (i.e. rostral ventrolateral medulla, RVLM) is sensitized by sodium, likely through the OVLT. This study (3) and others (115, 135), have found that HS leads to exaggerations in pressor reflexes, even in salt resistant rat models. This exaggeration in pressor reflexes causes augmented blood pressure variability (115, 135). Given this effect is present in normotensive salt resistant rat models, it is conceivable that this pathway would be present and exaggerated in rodent models of salt sensitive hypertension.

1.6.3.2 Central Nervous System in Salt Sensitive Hypertension

The DSS rat strain experiences hypertension and an exaggerated increase in the sodium content of cerebrospinal fluid when consuming a high sodium diet (53). This study also found that direct infusion of sodium into the right lateral cerebroventricle causes exaggerations for BP and renal sympathetic nervous activity responsiveness in the DSS strain (53). Interestingly, lesioning the AV3V region of the brain attenuates the development of hypertension in response to dietary sodium in the DSS rat (40). This suggests that the pathway previously discussed in salt resistant rats (see section 1.6.3.1), or a similar pathway in the same brain region, is crucial to the full development of salt sensitive hypertension. Kawano et al. (62) studied hypertensive humans and found that BP and the sodium content of cerebrospinal fluid were greater on a high sodium diet. However, the difference in cerebrospinal fluid sodium content between groups separated based on SS, was not significant (62). It is unclear if this discrepancy between studies represents a species difference or is due to previously established hypertension the population studied by Kawano et al (62). Studies

examining the central nervous system of normotensive adults in relation to their SS status are currently lacking.

1.6.3.3 Central Nervous System and Salt Sensitivity in Normotensives

Although there is much work yet to be done in the central nervous system model of SS, it is plausible that a link exists between the sodium within the blood/cerebrospinal fluid, central sensory mechanisms, and SS status in normotensives. Given the animal literature on the effects of sodium consumption on the central nervous system control of BP, it is likely that similar pathways exist in humans. It is also conceivable that an alteration within any link of this pathway could be responsible for the heterogeneous response to dietary sodium found among normotensive adults. A likely location for this alteration would be the RVLM, the primary controller of efferent sympathetic activity. Indeed, direct RVLM stimulation with glutamate microinjections in DSS rats with blood pressure controlled via a 0.3% salt diet display BP responses more than threefold higher than DSR rats (59). Therefore, if elevated SS status among normotensive humans is indeed driven by the RVLM, it is likely that such an individual would be hyperactive to several stimuli, not just dietary sodium. This suggests a need to study BP and sympathetic responsiveness relative to SS status to investigate the role of central drive in elevated SS among normotensive adults.

1.7 Blood Pressure and Sympathetic Responsiveness to Various Stressors in Humans and Animals with Prominent Salt Sensitivity

1.7.1 Psychological Stressors

In DSS rats, studies have shown exaggerated BP and sympathetic responses to air jet stress following a high sodium diet (54) and sodium infusion into the cerebrospinal fluid (53, 55), but not a regular sodium diet (54, 55). Unlike the studies in rodents, normotensive humans with high degrees of salt sensitivity consuming habitual sodium intake have exaggerated BP and heart rate variability responses to mental stress (13, 28, 29, 129), and enhanced startle modulation suggesting a role for the central nervous system in these responses (12). The differences between these studies during *typical* sodium consumption (i.e. regular sodium for rodents and habitual sodium for humans), may be the result of species differences, but is more likely due to the high intake typically consumed by humans in industrialized countries. With this in mind, these studies strongly suggest exaggerations in blood pressure and autonomic nervous system responsiveness to mental stress with high levels of SS during sodium consumption above recommended levels.

1.7.2 Physical Stressors

Results examining blood pressure and nervous system responsiveness to physical stressors are less clear. Using an anesthetized rat model, non-hypertensive DSS rats fed a normal sodium diet responded with exaggerated BP responses to sciatic nerve stimulation (64) compared to DSR rats. This study showed for the first time that phenotypic differences exists between DSS and DSR rats for BP responses to peripheral reflex stimulation similar to that experienced during muscle contraction. Importantly this difference was in the absence of hypertension and without a high salt

diet. This resembles the response found by Ito et al. (59) where direct RVLM stimulation with glutamate caused an exaggerated BP response in DSS rats during a 0.3% salt diet. To date, no human studies have examined the effects of SS on responsiveness to physical stressors in normotensive humans. Two human studies have examined the effects of SS on the responses to physical stressors in hypertensive humans. Omvik et al. (97) examined hemodynamic variables during leg cycling in adults with severe hypertension separated by SS status. The authors did not find statistically significant differences between groups with any cardiac or hemodynamic variable during rest or peak exercise. Campese et al. (15) measured plasma norepinephrine after 5, 10, 15 and 20 minutes of standing, and 40 minutes of ambulation during both low and high sodium diets in hypertensive humans relative to SS. They found greater increases in plasma norepinephrine during both standing and ambulation suggesting hyper-responsiveness of the sympathetic nervous system with elevations in SS. While not conclusive, the previous literature in animal and human models suggest high SS may be associated with exaggerated responsiveness to acute physical stressors.

Further research is needed to determine if peripheral reflex stimulation causes exaggerated BP responses in normotensive humans with high degrees of SS. If examinations of multiple tests of peripheral reflex stimulation show consistent exaggeration of BP and/or autonomic nervous system responsiveness, this lends support to the central nervous system model of SS. The exact mechanism for the potential augmentation of BP reactivity would be somewhat speculative within a human model. This is because such experimental methods as induced brain lesions are not feasible in human research. However, two mechanisms exist that may explain

such a finding: generalized augmented excitability of the RVLM and/or decreased baroreflex modulations of BP during excitatory maneuvers.

1.8 RVLM Excitability and Salt Sensitivity

The RVLM is the key brain region for controlling sympathetic outflow to the body. Blocking the activity of excitatory amino acids like L-glutamate with kynurenic acid injections into the RVLM cause's significantly greater decreases in BP in DSS vs. DSR rats on a low sodium diet; an even greater decrease in BP is displayed with kynurenic acid administration during HS in the DSS rat. This means it is possible that the DSS phenotype may be at least partially caused by greater RVLM excitatory responsiveness to HS. The augmented RVLM responsiveness would likely be due to greater tonic activity of excitatory amino acids. If this hypothesis is correct it would result in greater BP reactivity to sympathoexcitatory tests during regular sodium intake in DSS rats like that seen during direct sciatic nerve stimulation (64), a test known to rely on excitatory amino acids within the RVLM (65). In the human model of SS, peripheral reflex stimulation can be used as a non-sodium based test to examine the central (i.e. RVLM) sympathoexcitatory reactivity during habitual sodium intake. Given the results from animal models, it seems plausible that responsiveness to peripheral reflex stimulation would be related to SS status in humans.

1.9 Baroreflex Modulation of Blood Pressure Reactivity in Salt Sensitivity

1.9.1 Normal Baroreflex Function and Its Importance in Pressor Reflexes

In an intact organism, it would be difficult to determine if potential differences in BP reactivity to peripheral reflex stimulation are due to inherent differences in RVLM reactivity or due to poor modulation of RVLM activity by the baroreflex.

Following an increase in BP, a properly functioning baroreflex would sense a stretch in the carotid sinuses and aortic arch sending an excitatory signal via the glossopharyngeal nerve and vagus nerve, respectively. This signal is received by the nucleus tractus solitarius (NTS), which sends excitatory projections to the caudal ventrolateral medulla (CVLM) (122). The CVLM then sends an inhibitory projection to decrease the tonic activity of the RVLM resulting in decreased sympathetic activity and BP. This reflex continually functions to modulate BP during rest, exercise, and peripheral reflex stimulation. Determination of baroreflex sensitivity would be crucial to the interpretation of peripheral reflex stimulation results.

1.9.2 Rodent Models of Salt Resistance and Salt Sensitivity and Baroreflex Function

In the salt resistant Sprague Dawley rat strain, HS consumption not only causes an increase in RVLM responsiveness to excitatory stimulation (3, 4), but an increase in responsiveness to inhibitory stimulation as well (4). Thus a balance is maintained regardless of dietary sodium consumption, and the baroreflex sensitivity would likely be normal or even increased. If baroreflex sensitivity is different depending on SS status, the seemingly balanced excitatory and inhibitory RVLM responsiveness of the Sprague Dawley rat may not be present in a model with greater SS. Indeed, sinoaortic denervation (i.e. baroreceptor denervation) will convert Sprague Dawley rats into a highly SS strain (98). Therefore, depressed baroreflex sensitivity would result in BP abnormalities which may mechanistically explain SS in some models.

Many rodent studies have displayed impaired cardiac (11, 35, 88, 90, 108) and sympathetic (39, 54, 82) baroreflex sensitivity in salt sensitive rat strains. Gordon and Mark (38) examined DSS and DSR rats consuming LS to remove the confounding

variable of blood pressure. Direct electrical stimulation of the afferent aortic depressor nerve displayed equal responses for BP, heart rate, and sympathetic nervous activity suggesting that group differences were not caused by differences in central integration or efferent nerve activity. Additional examination of aortic arch distensibility was not different between rat strains. However, multifiber afferent aortic baroreceptor discharge following a phenylephrine induced increase in BP displayed less reactivity in the DSS strain. These studies suggest baroreflex function is depressed in the DSS rat strain regardless of dietary sodium or BP status due to less responsive stretch receptors within the arteries.

1.9.3 Human Salt Sensitivity and the Baroreflex

In a study on hypertensive humans, cardiac baroreflex sensitivity and heart rate variability were progressively lower as the level of SS increased regardless if they were consuming a HS or LS diet (19). This suggests lower parasympathetic and inhibitory nervous responses as SS increases. A single study has been conducted examining the baroreflex control of sympathetic activity in humans within the context of SS (123). This study found no differences for baroreflex function on either habitual or LS conditions between hypertensives grouped as “salt sensitive” or “salt resistant”. However, this study was likely underpowered with an n=11, only four of which were considered “salt resistant”. Therefore the available literature on SS and baroreflex control in humans is scarce. Furthermore, no human studies have been done to examine the baroreflex in the context of SS without the presence of hypertension. Additionally, no study has examined the baroreflex and SS during peripheral reflex stimulation such as the metaboreflex, which is known to increase baroreflex gain (44, 96). If peripheral reflex stimulation results in augmented BP reactivity with elevated

SS, and baroreflex gain is lower with increased SS, then the augmented responsiveness is likely partially due to a lack of BP restraint by the baroreflex. However, if the baroreflex is not lower with greater SS during peripheral reflex stimulation, then the augmented reactivity would have to be the result of another mechanism, such as generalized over reactivity of the RVLM. Research in this area is warranted as it may shed light on both mechanistic causes of greater SS, and poorer survival outcomes for adults with greater SS (130).

1.10 Testing Responses to Peripheral Reflex Stimulation

Studies in humans with elevated SS are needed to determine if the central nervous system responds abnormally to peripheral input. If examinations of multiple tests of peripheral reflex stimulation show consistent exaggeration of BP responsiveness, this lends support to the central nervous system model of SS.

Static handgrip is an ideal pressor test because of its relevance to daily life (e.g. griping objects such as a brief case, luggage, or groceries), and because it can be examined by its multiple pressor reflexes during experimental isolation from exercise and each other (5, 6, 21, 22, 26, 49, 80, 128). These reflexes are the metaboreflex and mechanoreflex.

1.10.1 The Metaboreflex

The metaboreflex is the pressor response brought about via the stimulation of nerve endings within the muscle by breakdown products of exercise. The metaboreflex is primarily initiated by group IV muscle afferent nerves (61). This reflex can be studied in isolation by occluding the blood flow to working muscle, and then having the participant cease the contraction. Doing so affectively maintains

metabolite stimulation of group IV fibers without continued muscle contraction; a technique often called post exercise ischemia (5, 26, 112).

1.10.2 The Mechanoreflex

The mechanoreflex, is the pressor response initiated by physical stretch or compression of nerve endings within the tissue and local blood vessels (61). This reflex can be tested with metabolite sensitization during post exercise ischemia by passively stretching the forearm (21). The mechanoreceptors of the forearm can also be tested in isolation during a venous distension protocol where blood flow to and from the forearm is stopped, and an infusion of isotonic saline through an intravenous catheter is used to stretch local veins initiating a pressor reflex (21, 23).

The pressor reflexes activated during muscle contraction are brought on by peripheral receptors which send signals to the brain. These signals are integrated in the control centers of the sympathetic nervous system, and propagated through the body by sympathetic activity resulting in a rise in BP. Additionally it is possible to test non muscle related reflexes such as cold exposure via the cold pressor test (127). Both the sympathetic nervous system and resulting BP can be measured within the intact human during all of these tests. Doing so provides insight into the function of the central nervous system. Therefore the results of a comprehensive study of various pressor reflexes in relevance to SS may help to answer questions regarding the central nervous system in this complex etiology. Additionally, such studies could provide insight into the clinically important link between SS and cardiovascular disease and mortality.

1.11 Consequences of Elevated Blood Pressure Reactivity to Peripheral Stimulation

Regardless of the mechanism, if BP responses are exaggerated to common tasks such as muscle contraction or cold exposure then it is possible that these exaggerated cardiovascular demands to routine tasks contribute to the poor survival of adults with high degrees of SS (130). Support for this was found in a recent study comparing DSS and DSR rats with sinoaortic denervation (2). This study found that the pressor reflex caused by throat muscle contraction during fluid consumption, a reflex thought to be driven by stimulation of mechanoreceptors (1), caused bradyarrhythmias during drinking in DSS but not DSR rats. Additionally the DSS rats displayed significantly higher absolute BP at rest and during drinking as well as more premature ventricular contractions during drinking (2). Large variations in BP as a result of such common activities may be captured with the use of 24hr ambulatory BP monitors, warranting such a measure made relative to SS.

1.12 Blood Pressure Variability

Only very short term (beat-to-beat) and short term (over 24hrs) BP variability are thought to be strongly influenced by the central nervous system (99). While no universally accepted norms exist for classification of short term BP variability status (18, 85, 103), increased standard deviation (SD) in 24 hour BP measurement with increases in mean BP have been found (normotensive SD ≈ 5 mmHg to severe hypertension SD ≈ 9 mmHg) (76, 78). Additionally, when matched for 24 hour BP, greater short term BP variability is linked with greater cardiac and vascular damage (77, 78, 100), and increased incidence of cardiovascular events (66, 75, 105, 126). Therefore, if elevated SS is shown to be coupled with elevated BP reactivity, then short term BP variability should be assessed to determine if it is also increased with

increases in SS. Data supporting a relationship between elevated SS and elevated BP variability has been shown by Chao et al. (17), but currently no peer reviewed publications have focused on this relationship.

1.13 Conclusion

Blood pressure responsiveness to dietary sodium is different between individuals, and is predictive of future hypertension, cardiovascular events, and death. The cause of this differential response termed, salt sensitivity, is likely multifactorial. Recent evidence supports a strong role for the central nervous system in determining an individual's salt sensitivity status. Abnormalities of the central nervous system may result in augmented blood pressure responsiveness to peripheral stimuli. This heightened responsiveness causes the cardiovascular system to be in a heightened state during routine activities, potentially accounting for the worsened health outcomes of adults with pronounced salt sensitivity. Future research is needed within this area to clarify the role of the central nervous system in determining salt sensitivity, and how potential alterations in central nervous system function in adults with high salt sensitivity may affect blood pressure reactivity to other stimuli (see figure 1.4).

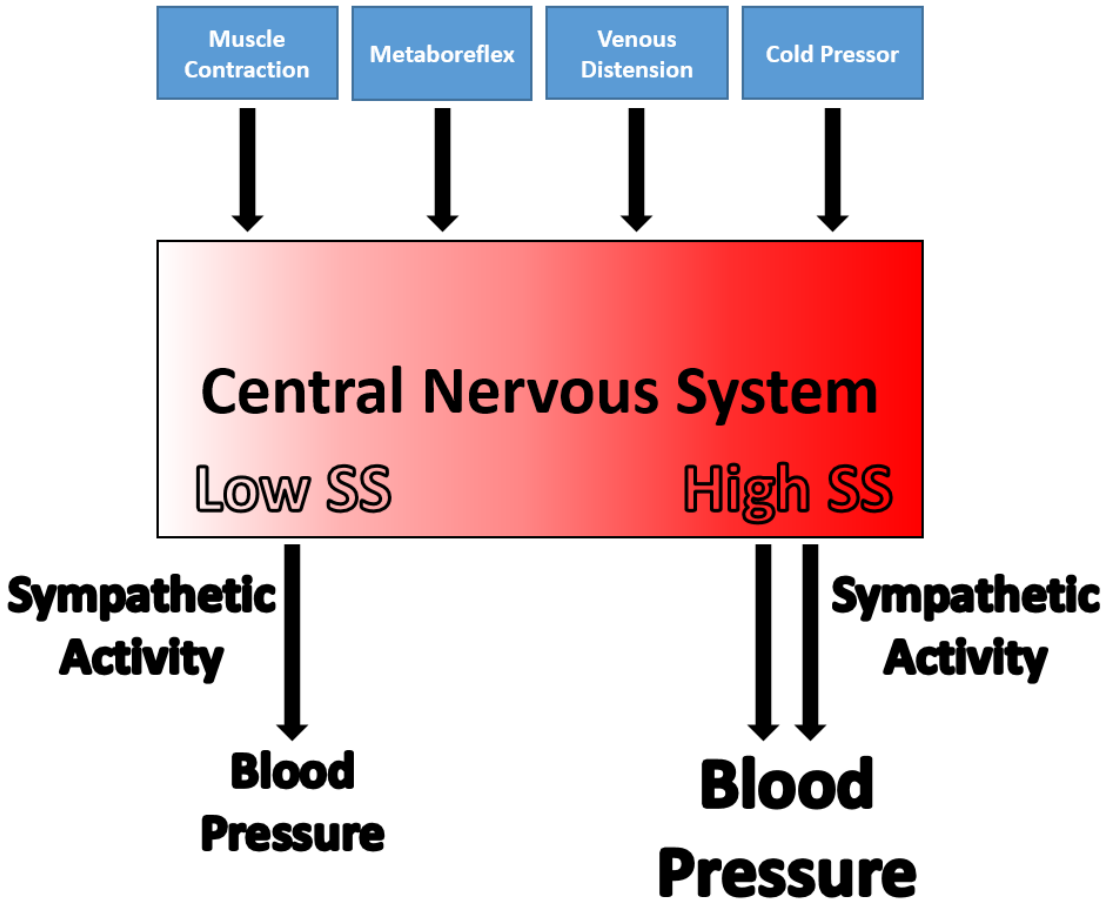

Figure 1.4 **Working Model Diagram.** Model displaying the potential for exaggerated peripheral reflex pressor responses to a variety of stimuli in normotensive adults with higher salt sensitivity (High SS) vs. lower salt sensitivity (Low SS).

Chapter 2

BLOOD PRESSURE RESPONSIVENESS TO SYMPATHOEXCITATORY TESTS IS NOT RELATED TO SALT SENSITIVITY STATUS IN NON-HYPERTENSIVE ADULTS

2.1 Introduction

The blood pressure (BP) response to manipulations of dietary sodium varies widely in both normotensive and hypertensive adults (132). This heterogeneity is termed salt sensitivity (SS). Studies examining SS have found clinically relevant relationships between SS status and future outcomes. Weinberger and Fineberg (131) found normotensive adults with high levels of SS had a greater increase in systolic BP and diastolic BP over time than those with less SS. Similarly it has been found that normotensive adults in the upper 50% for SS had 37.5% higher prevalence of hypertension after a 15 year follow up (8). This relationship to hypertension development is important because cardiovascular disease is the leading cause of death in the United States (85). In fact, non-hypertensive humans with elevated SS have poor survival rates similar to hypertensives (130). The determinants SS status are still under investigation.

In recent years the central nervous system has been identified as a potential determinant of SS status. The rostral ventrolateral medulla (RVLM) within the brain is a particularly important area due to its significant role in controlling the outflow of sympathetic activity and therefore BP. Ito et al. (59), found that blocking excitatory amino acids within the RVLM with kynurenic acid microinjections caused a large and immediate drop in blood pressure (40±2 mmHg) in Dahl salt sensitive (DSS) rats, but not Dahl salt resistant (DSR) rats during high dietary sodium (HS) intake, suggesting a potential role for the RVLM in determining SS status. Interestingly, blockade also

lowered blood pressure (16±2 mmHg) in the DSS rat during low dietary sodium (LS) without effecting the DSR rat, suggesting central nervous system dysfunction regardless of sodium intake in the DSS rats. Given the important role of the RVLM in reflex sympathoexcitation, it is then plausible that abnormalities in this region would result in exaggerated pressor responsiveness. Indeed, direct RVLM stimulation with glutamate microinjections in DSS rats during LS (0.3% salt) resulted in BP responses more than threefold higher than DSR rats (59), confirming DSS rats have over responsive RVLM regions. Therefore, it seems reasonable to ask if differences exist in the sympathoexcitation to peripheral pressor reflexes in animals and humans with elevated SS. Kenney et al. found that in non-hypertensive DSS rats consuming a normal sodium diet, the BP response to sciatic nerve stimulation is exaggerated (64). This study showed for the first time that there is a phenotypic difference between DSS and Dahl salt resistant (DSR) rats for BP responses to peripheral reflex stimulation. Such a hypothesis has never been tested in humans before.

If BP responsiveness is exaggerated to multiple peripheral reflex stimulation tests in humans with high degrees of SS, the commonality of these reflexes (i.e. the RVLM within the central nervous system) is likely the mechanism of this dysfunction. Furthermore, this would provide evidence prompting questions for future research investigating alterations in RVLM function as a potential mechanism for high SS in non-hypertensive humans. Additionally, it is possible that these proposed exaggerated responses would result in exaggerated cardiovascular demands to routine tasks, and contribute to the poor survival of adults with high degrees of SS (130). Therefore, the aim of this analysis is to determine the neurocirculatory responses to a variety of tests causing peripheral reflex stimulation. Doing so will provide a peripheral window into

the central nervous system of individuals with high degrees of SS. We hypothesize that blood pressure and nervous system responses to peripheral reflex stimulation will be positively related to SS status.

2.2 Methods

2.2.1 Participants and Visit Schedule

All procedures and protocols utilized are in adherence to the Declaration of Helsinki of 1975, as revised in 1983, and were approved by the Institutional Review Board (IRB) of the University of Delaware. Leading up to the data collection visit participants underwent a series of visits and measurements (see figure 2.1). All participants were first recruited from the surrounding community in and around Newark, DE, USA, and represented the sexual and racial makeup of Delaware, USA.

2.2.1.1 Initial Screening Visit

Participants underwent an initial screening visit at the Nurse Managed Health Center at the University of Delaware prior to participation in a controlled diet SS assessment (see figure 2.1). During the initial screening visit participants signed an IRB approved consent in order to participate in the SS assessment. During this screening the following tasks were performed: medical history questionnaire, physical activity readiness questionnaire, resting blood pressure, resting 12-lead electrocardiogram, height measurement, weight measurement, and blood sample. All participants were between 22-60 years old at the time of the initial screening. At this time participants were excluded for the following criteria: history or evidence of cancer, diabetes, heart disease, hypertension, any other chronic disease, hormone replacement therapy, tobacco or nicotine use, or body mass index greater than 30.

2.2.1.2 Salt Sensitivity Assessment

SS was assessed utilizing a 21 day controlled feeding study consisting of a 7 day run-in period (100 mmol Na+/d) followed by a two phase randomized crossover 7-day diet perturbation: low sodium (LS); 20 mmol Na+/d and high sodium (HS); 300 mmol Na+/d (see figure 2.1). Twenty-four hour urine collections were done on the final day of the HS and LS phases and analyzed for sodium excretion to confirm participant adherence to the feeding protocol. BP was assessed on the final day of the LS and HS phase by 24hr ambulatory BP monitors (Model 90207; Spacelabs Medical, Issaquah, WA, USA) (see figure 2.1). The 24hr BP monitor was programmed to measure BP every 20 minutes during the day and every 30 minutes while sleeping. SS was calculated as the change in 24hr mean arterial BP (MAP), 24hr MAP HS – 24hr MAP LS.

2.2.1.3 Second Screening Visit and Pre-Testing Measurements

Following the SS assessment a second recruitment effort was made to enroll participants in the experiments related to the current investigation. Prior to participating in the planned experiments, participants underwent a second screening visit. The second screening visit took place in the Cardiovascular Research Laboratory at the University of Delaware. During this screening visit, participants signed a second IRB approved consent to enroll in the remainder of the study protocols. The purpose of the second screening visit was to confirm that there are no major changes in health or medication/drug usage status since the SS assessment. During this screening, the following tasks were performed: medical history questionnaire, physical activity readiness questionnaire, resting blood pressure, resting electrocardiogram assessment, height measurement, and weight measurement. All

results from the second screening visit were reviewed by the study nurse practitioner to determine participant eligibility. Additionally, participants filled out a menstruation questionnaire to provide insight into the hormone levels of the female participants. This was used to ensure that premenopausal women were studied during their low hormone phase, typically the first five days of menstruation.

Immediately prior to the data collection visit, participants performed a 3 day diet record and 24hr urine collection to assess habitual sodium intake (see figure 2.1). The 24hr urine collection was analyzed for urine volume, electrolytes (Easy Electrolyte Analyzer; Medica, Bedford, Massachusetts, USA), and osmolality (Advanced 3D3 Osmometer; Advanced Instruments, Norwood, Massachusetts, USA). Free water clearance, and fractional sodium, chloride and potassium excretion were determined. Results from the 3 day diet record and 24hr urine collection were similar for sodium intake/excretion.

An additional 24hr ambulatory BP assessment was done to examine the habitual BP profile (see figure 2.1). The standard deviation of the MAP during the habitual sodium consumption was used as a measure of short term BP variability as this is largely under central nervous system control (99) and is predictive of vascular and cardiac damage (77, 78, 100). All participants reported to the laboratory the day of the data collection visit after avoiding alcohol, caffeine, and exercise for 24hrs, and fasting for 4 hours.

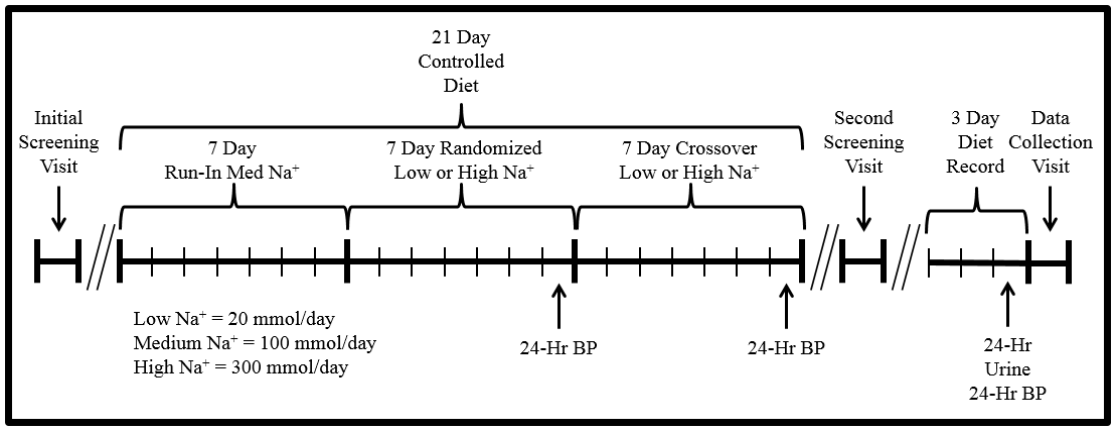

Figure 2.1: **Depiction of Participation Timeline.** All participants were initially screened to exclude individuals with chronic conditions from undergoing salt sensitivity assessment. The salt sensitivity assessment involved a three week dietary feeding protocol. The first week all participants consumed the recommended daily intake of sodium. Weeks two and three were a randomized crossover design providing low and high dietary sodium for one week each. Twenty-four hour mean arterial blood pressure was performed on the final day of the low and high sodium weeks for use in salt sensitivity calculation (high sodium 24hr MAP – low sodium 24hr MAP). Participants were given a minimum of 1 month from the end of the salt sensitivity assessment to allow a return of habitual sodium intake. Participants then underwent a second screening visit to insure no change in health status, and to perform normal screening related measurements (e.g. blood pressure, heart rate, etc.). Immediately prior to the data collection visit, participants performed a three day diet record and 24hr urine collection to assess habitual sodium consumption. All sympathoexcitatory tests were performed during a single data collection visit. BP, blood pressure; MAP, mean arterial pressure.

2.2.2 Experimental Measurements

Participants were tested in the supine position with their head and left leg supported slightly above the plane of the body. The non-dominant hand was held at the level of the heart with arm juxtaposed to the torso, while the dominant arm was placed at approximately a 30° horizontal angle from the torso. Beat-by-beat arterial BP was measured from the middle finger of the non-dominant hand using a servo-controlled finger photoplethysmographer (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands) (see figure 2.2). Manufacturer recommendations were followed for calibrating the finger pressure to brachial artery pressure. As done elsewhere (87), finger pressures were level corrected post hoc to match the baseline finger pressure to baseline brachial artery pressure (Dinamap Dash 2000; GE Medical Systems, Milwaukee, WI, USA) to verify satisfactory absolute Finometer BP measures. Electrocardiograph lead II (Dinamap Dash 2000; GE Medical Systems, Milwaukee, WI, USA) was monitored to determine heart rate (HR). Respiratory movements at the abdomen and chest were monitored throughout trials using a strain-gauge pneumograph to insure that the participants maintained normal breathing patterns free of Valsalva maneuvers. Ratings of perceived exertion (RPE) using the 6-20 Borg scale (9) was used throughout handgrip exercise.

Muscle sympathetic nervous activity (MSNA) measurement was done via microneurography (121) (see figure 2.2). This technique uses a primary tungsten recording microelectrode inserted in the peroneal nerve behind the fibular head. A second reference microelectrode was inserted 2-3 cm from the primary recording microelectrode. The nerve signal was amplified (factor = 70,000), bandpass filtered (700-2,000 Hz), rectified, and integrated (time constant 0.1 s) using a nerve traffic analyzer (Nerve Traffic Analyzer, model 662c-3; University of Iowa Bioengineering,

Iowa City, IA, USA). Multiple criterion were used to confirm that the nerve signal obtained was MSNA rather than skin sympathetic nervous activity. Confirmation of MSNA required the following: absence of an increase in afferent activity during light stroking of the skin, increased efferent burst frequency during voluntary end-expiratory apnea, and observable spontaneous cardiac cycle gaiting of efferent bursts. The preprocessed MSNA signal was analyzed using a custom designed LabVIEW program (32) which generates synchronized beat-by-beat data of MSNA, BP, and HR. The program identifies bursts of MSNA using an R-wave gating approach. The mean value of the three largest bursts is assigned a value of 100 arbitrary units (AU); after which all bursts are scaled accordingly. MSNA values are reported as burst frequency (burst per unit of time normalized to 1 minute, bursts/min), burst incidence (bursts/ 100 heart beats), total MSNA (burst height [AU]/heart beat) and total activity (burst frequency • mean burst height, AU/min).

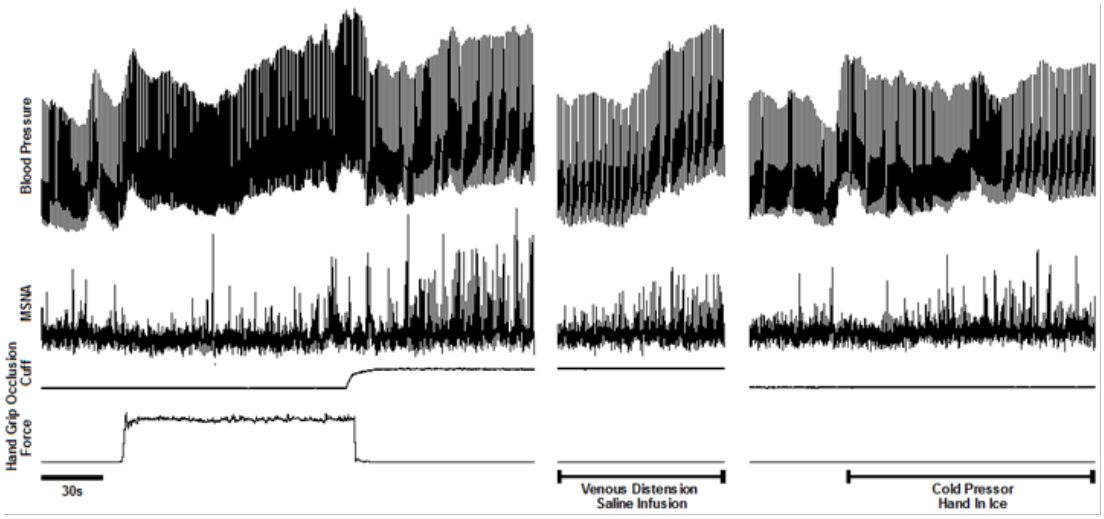

Figure 2.2: **Example Tracing of Key Study Variables during Various Trials.** Continuous measures from top to bottom: beat-by-beat blood pressure, muscle sympathetic nervous system activity, arterial occlusion cuff pressure, and handgrip force. Trials from left to right: handgrip immediately followed by post exercise ischemia, venous distension, and cold pressor test. MSNA, muscle sympathetic nervous activity.

Limb vascular resistance was indexed using a methodological approach blended from multiple previous studies (32, 102, 107). Duplex Doppler ultrasound (Logiq e or GE P5; GE Healthcare, Waukesha, Wisconsin, USA) was held by a clamp on the upper thigh 2 to 3 cm proximal to the bifurcation point of the superficial and deep femoral arteries at a 60° angle to the common femoral artery (CFA). CFA blood flow was measured in 5 consecutive heart beats during each 30s interval and used as an index of blood flow for that time segment. CFA blood flow was calculated as [time averaged mean blood velocity (cm/s)] • π • [3 measurement mean diastolic diameter (cm) / 2]^2 • 60 s/min (32). CFA vascular resistance index was calculated as [30s average MAP] / [CFA blood flow] and was expressed as a change relative to baseline (107).

An intravenous catheter (IV) was inserted into the antecubital space of the dominant arm in a retrograde fashion. The IV was used to draw blood and to infuse isotonic saline during the venous distension trial. Venous blood samples were analyzed to determine hemoglobin (Hb 201+ model; HemoCue, Lake Forest, California, USA), hematocrit (Clay Adams Brand, Readacrit Centrifuge; Becton Dickinson, Sparks, Maryland, USA), serum electrolytes (Easy Electrolyte Analyzer; Medica, Bedford, Massachusetts, USA), and plasma osmolality (Advanced 3D3 Osmometer; Advanced Instruments).

2.2.3 Experimental Trial Protocols

2.2.3.1 Isometric Handgrip

The participant's maximum voluntary contraction (MVC) of the dominant hand was measured by a grip force transducer (MLT004/ST Grip Force Transducer, ADInstruments, Colorado Springs, Colorado, USA). Three to five maximal attempts separated by at least 60s was made with the highest recorded as the MVC. Participants performed 2 minutes of constant isometric handgrip at 40% MVC (see figure 2.2). Participants were given visual feedback of their force production throughout the handgrip trial. The final 30s of exercise was analyzed and expressed relative to the period of rest leading up to muscle contraction as a change from baseline.

2.2.3.2 Post Exercise Ischemia

Just prior to cessation of muscle contraction, (2 minutes of handgrip at 40% MVC), an occlusion cuff placed around the upper arm was inflated to 240 mmHg (Rapid Cuff Inflator; Hokanson, Bellevue, Washington, USA) trapping the metabolites

created during contraction (see figure 2.2). This period of PEI was maintained for 3 minutes and 15s after the end of muscle contraction. The final 30s of PEI was analyzed and expressed as a change relative to the period of rest leading up to muscle contraction.

2.2.3.3 Venous Distension

Venous distension was performed utilizing an IV inserted in an antecubital vein of the dominant arm. The arm was momentarily elevated to a vertical position and wrapped with an elastic bandage to partially exsanguinate the arm. An occlusion cuff placed around the upper arm was then inflated to 240 mmHg (Rapid Cuff Inflator; Hokanson, Bellevue, Washington, USA). Following cuff inflation, the forearm wrapping was removed and the arm was returned to its resting position. Two minutes later, infusion was initiated of a 0.9% NaCl saline solution. The total volume infused was equal to 5% of forearm volume (20), as estimated based on forearm length and girth measurements (43). The infusion period lasted approximately 90s (see figure 2.2), while the total occlusion time was 9 minutes. The venous distension response was calculated as the change from baseline to the 30s immediately following infusion (peak BP response) (20).

2.2.3.4 Cold Pressor Test

The cold pressor test was performed by submerging the participants dominant hand in ice water for 2 minutes (127) (see figure 2.2). The response to the cold pressor test was calculated as the change from baseline to the final 30s of cold exposure.

2.2.4 Data Acquisition and Analysis

All measurements collected during the data collection visit, except for those made via ultrasound, were recorded at 1,000 Hz using the PowerLab data acquisition system and analyzed with LabChart 7 software (ADInstruments, Colorado Springs, Colorado, USA), or a custom designed LabVIEW program (32). The time segments and length of time segments (i.e. 30s) analyzed for each trial were chosen to capture the peak BP response. Although time segments for peak response were 30s in length, values are presented per minute (value for 30s segment multiplied by 2) to express results in commonly reported units.

2.2.5 Statistical Analysis

All variables examined relative to SS were analyzed by linear regression analysis. Single sample t-tests were used to determine if response values (delta scores) were different from zero. All variables regressed over SS were also tested using multiple regression to confirm relationships independent of age. Additionally, exploratory analysis was performed examining men only and women only for all linear regression analyses. Results were similar between sexes suggesting the findings of the group as a whole were not affected by examining men and women together. Therefore, sex specific results are not shown in the results section. Exploratory analysis was also performed using various BP measures to assess SS to be sure the primary conclusions were not affected by the limited range found using 24 hour mean arterial pressure (MAP). This secondary analysis did not affect the primary conclusions. Consequently, it is not shown in the results section. Alpha level of significance was set at $p<0.05$ for all statistical tests. Values expressed as mean ± SEM.

2.3 Results

2.3.1 Participants

Fifty participants were recruited for the current analysis. All participants underwent SS assessment. Two participants withdrew prior to the data collection visit. Two additional participants were excluded based on a lack of an increase in sodium excretion during the HS diet vs the LS diet (<100Δmmol/24hr). The data represented in the current analysis represents the data collected from the remaining 46 participants. The average time between completion of the SS assessment and the enrollment in the current study was 237±28 days, and exhibited no relationship with SS status (r=0.05; p=0.732). Participant characteristics are displayed in table 2.1. SS was directly related to age (r=0.34; p=0.021). No other screening or baseline biochemical parameters were related to SS. Twenty-four hour SBP (118±1mmHg, r=-0.334, p=0.087), DBP (72±1mmHg, r=-0.099, p=0.624), MAP (87±1mmHg, r=-0.004, p=0.986) were not related to SS status. Twenty-four hour pulse pressure (46±1mmHg, r=-0.442, p=0.020) was related to SS status in that those with the highest SS had the lowest pulse pressure. The BP dip the occurred while sleeping was not related to SS status for SBP (11.7±1.2%, r=-0.196, p=0.348), DBP (16.3±1.3%, r=-0.351, p=0.084), MAP (12.7±1.2%, r=-0.299, p=0.145). Twenty-four hour BP standard deviation was inversely related to SS status for SBP (11.7±0.5mmHg, r=-0.380, p=0.0498), but not DBP (9.7±0.4mmHg, r=-0.208, p=0.296) or MAP (9.6±0.4mmHg; r=-0.15; p=0.453) were not related to SS.

Table 2.1: Participant Characteristics

Screening Demographic Data	Mean ± SEM	SS r Value	SS p Value
N (Sex Coded: M1,F2)	46 (25/21)	0.21	0.152
Race (Coded: W1, O2, B3)	W39, O2, B5	0.22	0.149
Age (Years)	38 ± 2*	0.34	0.021
Height (cm)	174 ± 1	-0.19	0.199
Mass (kg)	73.1 ± 1.9	-0.07	0.644
BMI (kg/m^2)	24.2 ± 0.5	0.06	0.716
SBP (mmHg)	114 ± 1	-0.11	0.448
DBP (mmHg)	68 ± 1	-0.14	0.343
MAP (mmHg)	83 ± 1	-0.15	0.324
Heart rate (Beats/min)	59 ± 1	0.16	0.296
Baseline Biochemical Parameters			
Hemoglobin (g/dL)	13.3 ± 0.2	-0.26	0.129
Hematocrit (%)	40 ± 1	-0.09	0.588
Serum Sodium (mmol/L)	138.1 ± 0.5	-0.08	0.623
Serum Potassium (mmol/L)	4.11 ± 0.06	0.19	0.263
Serum Chloride (mmol/L)	104.4 ± 0.4	0.00	0.988
Plasma OsM (mOsm/kg H_2O)	287 ± 1	-0.20	0.260
Urine Sodium (mmol/24hr)	165.5 ± 9.0	-0.10	0.506
Urine Potassium (mmol/24hr)	73.7 ± 4.2	0.09	0.576
Urine Chloride (mmol/24hr)	197.1 ± 7.2	-0.03	0.848
Urine OsM (mOsm/kg H_2O)	886.1 ± 27.9	-0.13	0.406
Urine Flow Rate (mL/min)	1.46 ± 0.09	0.17	0.265
Free Water Clearance (mL/min)	-0.73 ± 0.11	0.23	0.185
Three Day Diet Record Values			
Total Energy (kcal/d)	2242 ± 70	-0.09	0.532
Sodium (mg/d)	3495 ± 147	-0.16	0.277
Potassium (mg/d)	2972 ± 126	0.10	0.522
Calcium (mg/d)	1137 ± 59	0.04	0.800

Participant demographic data was collected during the screening visit. Urine was collected over the 24 hours prior to data collection visit. Blood was collected at the data collection visit prior to any trial. The three day diet record data was collected during the three days immediately prior to the data collection visit. Salt sensitivity was assessed by individually examining the increase in 24hr ambulatory mean arterial pressure during the high dietary sodium diet compared to the low dietary sodium diet during the salt sensitivity assessment. SS, salt sensitivity; M, male; F, female; W, white; B, black; O, all other; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; OsM, osmolality. * $p<0.05$ relation to salt sensitivity.

2.3.2 Peripheral Reflex Stimulation Trials

Baseline characteristics from the data collection visit are displayed in table 2.2. RPE increased during the handgrip trial ($p<0.001$). No relationship exists between SS and handgrip MVC, peak RPE, venous distension saline volume, or venous distension infusion rate. Baseline BP, HR, and femoral artery characteristics: blood velocity, diameter, blood flow, and resistance, were unrelated to SS. Baseline MSNA is related to SS reaching statistical significance for burst frequency ($r=0.47$; $p=0.041$) and burst incidence ($r=0.46$; $p=0.046$). Multiple regression analysis did not result in SS being independent of age as a predictor of resting MSNA (all $p>0.05$). The inflation of the occlusion cuff during the venous distension trial did not result in a change in BP, HR, or MSNA (all $p>0.05$), therefore all venous distension responses are presented relative only to the venous distension baseline prior to the inflation of the occlusion cuff.

Table 2.2: Baseline Characteristics

Trial Characteristics, n	Mean ± SEM	SS r Value	SS p Value
Handgrip Trial, n=46			
MVC (N)	233 ± 14	-0.24	0.111
Peak RPE	14 ± 0.4	-0.04	0.778
Post Exercise Ischemia, n=45			
Blood Lactate (∆mmol/L)	1.48 ± 0.15	-0.31	0.080
Blood pH (∆)	-0.066 ± 0.017	-0.08	0.665
Serum Potassium (∆mmol/L)	1.10 ± 0.06	-0.09	0.643
Venous Distension, n=32			
Saline Volume (mL)	50 ± 2	0.01	0.954
Infusion Rate (mL/min)	35 ± 4	0.12	0.527
Blood Pressure/HR, n=46			
SBP (mmHg)	116 ± 1	-0.02	0.893
DBP (mmHg)	75 ± 1	0.06	0.698
MAP (mmHg)	93 ± 1	0.04	0.797
HR (Beats/min)	60 ± 1	-0.07	0.666
Femoral Artery Ultrasound, n=45			
TAmean Blood Velocity (cm/s)	9.48 ± 0.82	-0.06	0.716
Diameter (cm)	0.92 ± 0.02	-0.05	0.761
Blood Flow (mL/min)	355 ± 28	-0.13	0.394
R Id (mmHg/mL/min)	0.307 ± 0.024	0.23	0.139
Sympathetic Nervous Activity, n=19			
Burst Frequency (Bursts/min)	20.2 ± 2.1*	0.47	0.041
Burst In (Bursts/100 Heart Beats)	34.2 ± 3.7*	0.46	0.046
Total MSNA (AU/Beat)	16.0 ± 1.7	0.44	0.058
Total Activity (AU/min)	950.1 ± 100.8	0.43	0.065

Baseline characteristics represent measurements recorded immediately prior to the first trial during the data collection visit. HR, heart rate; SS, salt sensitivity; MVC, maximal voluntary contraction; Peak RPE, peak rating of perceived exertion; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; TAmean, time averaged mean; R Id, resistance index; Burst In, burst incidence; Total MSNA, total muscle sympathetic nervous activity, AU, arbitrary units.

* $p<0.05$ relation to salt sensitivity.

2.3.2.1 Isometric Handgrip

Isometric handgrip exercise caused an increase in BP (all $p<0.05$) that was not related to SS (see figure 2.3 A-C). The increase in HR ($p<0.001$) with handgrip exercise was inversely related to SS in that greater SS was associated with a smaller increase in HR ($r=-0.33$; $p=0.025$; see figure 2.4 D). Multiple regression analysis did not result in SS being independent of age as a predictor of handgrip induced HR response ($p=0.178$). Handgrip induced an increase in all measures of sympathetic activity (all $p<0.05$). The MSNA response as measured by MSNA burst frequency ($r=-0.50$; $p=0.032$), total MSNA ($r=-0.54$; $p=0.024$), and total activity ($r=-0.65$; $p=0.004$) were all inversely related to SS in that those with the greatest SS exhibited the lowest increase in sympathetic activity (see figure 2.4 A, C, D). Multiple regression analysis did not result in SS being independent of age as a predictor of handgrip induced MSNA burst frequency ($p=0.057$), but SS was an independent predictor of total MSNA ($p=0.014$), and total activity ($p=0.003$). The burst incidence response was not significantly related to SS ($r=-0.37$; $p=0.129$; figure 2.4 B). Handgrip induced an increase in femoral artery blood velocity and blood flow (both $p<0.05$), but not diameter ($p=0.495$). Group femoral artery resistance index tended to increase, but did not reach statistical significance ($p=0.094$). The change values for femoral artery blood velocity and diameter were not related to SS status (both $p>0.05$; see figure 2.5 A-B). Handgrip responses for common femoral artery blood flow were directly related to SS with a greater increase in blood flow in those with greater SS ($r=0.33$; $p=0.036$; see figure 2.5 C), and SS was independent of age ($p=0.045$). The change values for femoral artery resistance index were inversely related to SS ($r=-0.38$; $p=0.016$; see figure 2.5 D), and SS was independent of age ($p=0.45$). Visual examination of the individual data show adults with the lowest SS largely displayed an

increase in resistance, while adults with the highest SS showed no change or a decrease in resistance.

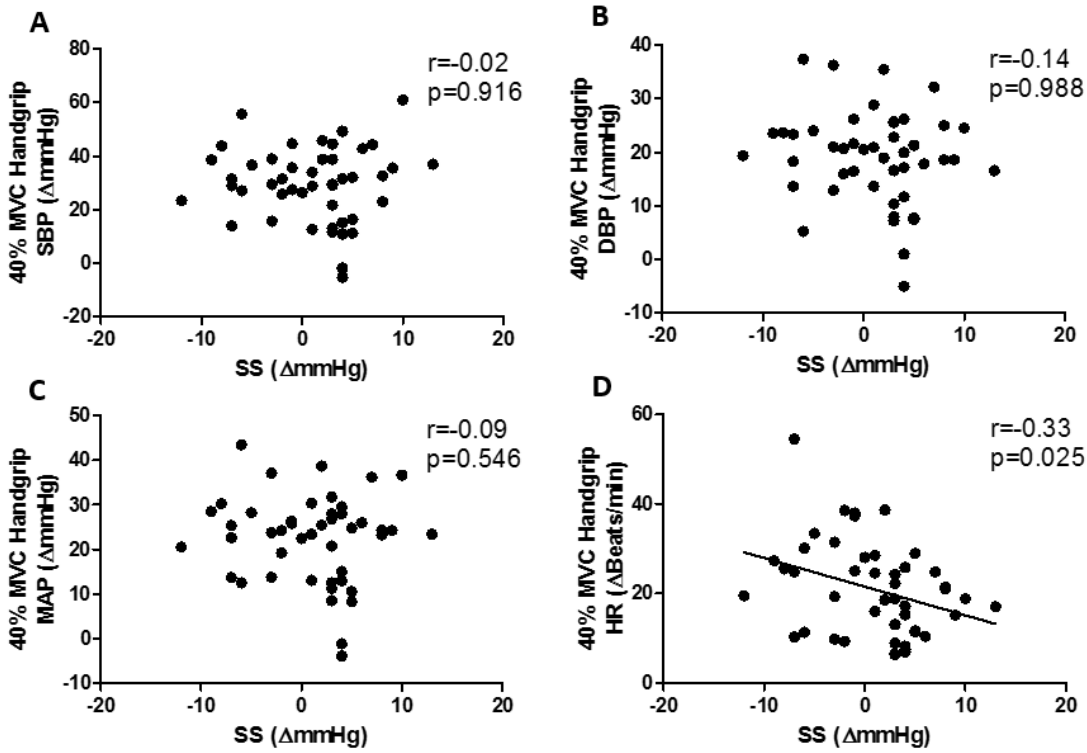

Figure 2.3 **Blood Pressure and Heart Rate Responses to Isometric Handgrip.** No relationship between the increase in systolic, diastolic, or mean arterial pressure was found with salt sensitivity. The increase in heart rate was inversely related to salt sensitivity. A. Change in systolic blood pressure. B. Change in diastolic blood pressure. C. Change in mean arterial pressure. D. Change in heart rate. SS, salt sensitivity; MVC, maximal voluntary contraction; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; HR, heart rate.

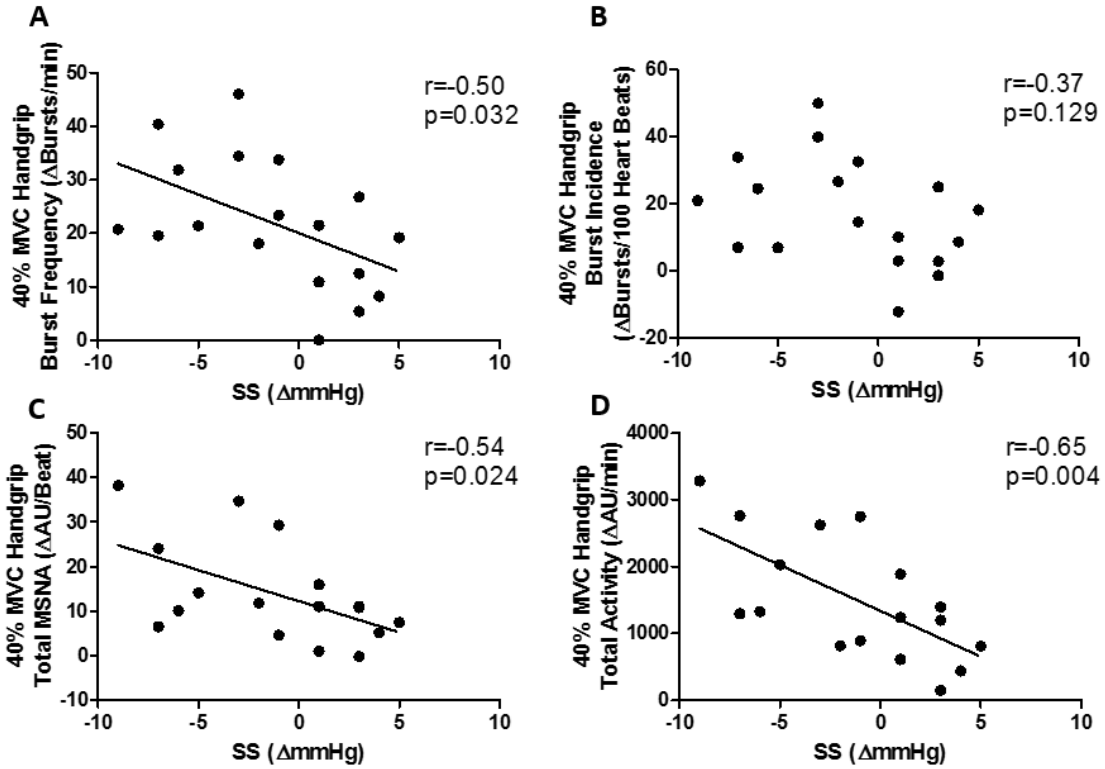

Figure 2.4 **Muscle Sympathetic Nervous Activity Response to Isometric Handgrip.** The increase in sympathetic activity is less with high levels of salt sensitivity for the measures of burst frequency, total MSNA, and total activity, but not burst incidence. A. Change in burst frequency. B. Change in burst incidence. C. Change in total MSNA. D. Change in total Activity. SS, salt sensitivity; MVC, maximal voluntary contraction; MSNA, muscle sympathetic nervous activity; AU, arbitrary units.

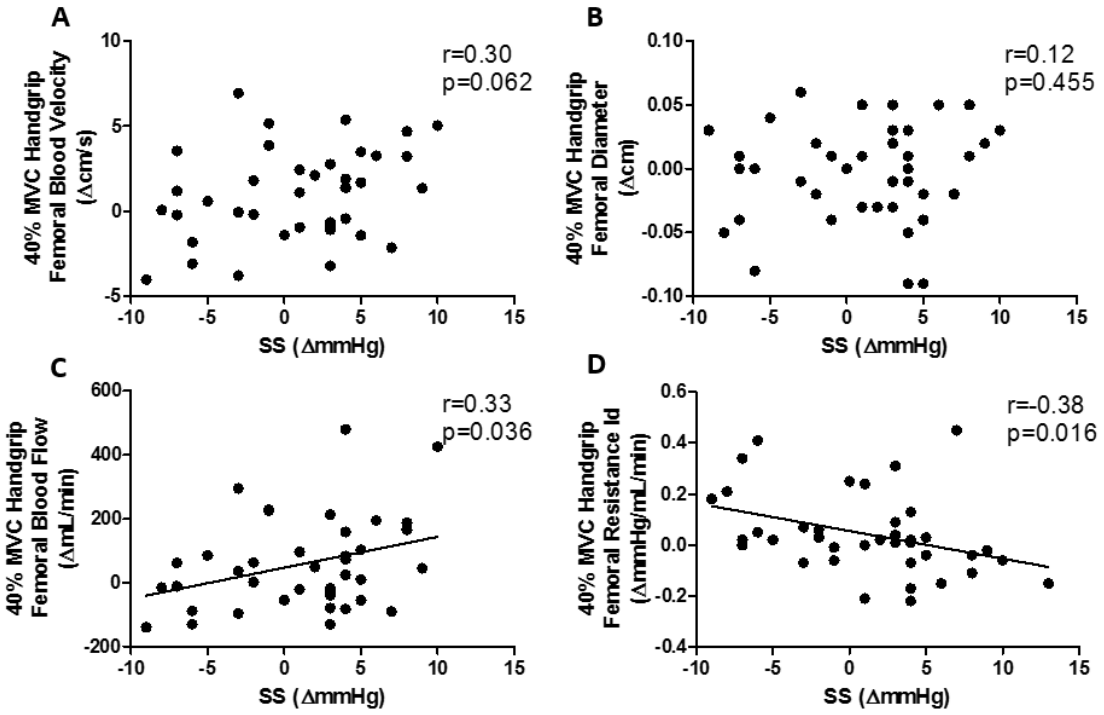

Figure 2.5 **Common Femoral Artery Responses to Isometric Handgrip.** No relationship exists between salt sensitivity and femoral blood velocity or diameter changes. The change in femoral artery blood flow was directly related to salt sensitivity, while femoral artery resistance was inversely related. A. Change in femoral artery blood velocity. B. Change in femoral artery diameter. C. Change in femoral artery blood flow. D. Change in femoral artery resistance index. SS, salt sensitivity; MVC, maximal voluntary contraction; Id, index.

2.3.2.2 Post Exercise Ischemia

PEI was performed in conjunction with the isometric handgrip trial by inflating an occlusion cuff on the upper arm ≈5s prior to the cessation of muscle contraction. BP, HR, MSNA, femoral blood velocity, and blood flow remained significantly elevated above baseline during PEI (all $p<0.05$), while femoral diameter and resistance index were not significantly greater than that during baseline (both $p>0.05$). No significant association was found between SS and the PEI induced changes in BP

(see figure 2.6 A-C), HR (see figure 2.6 D), sympathetic activity (see figure 2.7), or femoral artery measures (see figure 2.8).

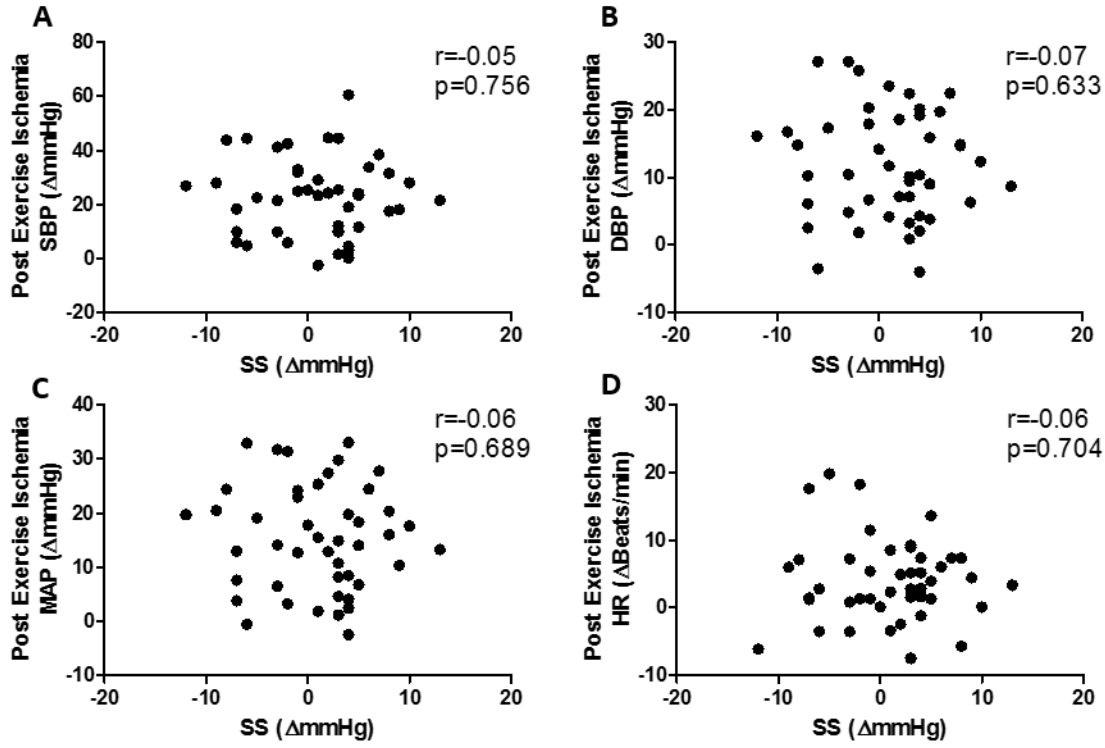

Figure 2.6 **Blood Pressure and Heart Rate Responses to Post Exercise Ischemia.** No relationship between the increase in systolic, diastolic, mean arterial pressure or heart rate was found with salt sensitivity. A. Change in systolic blood pressure. B. Change in diastolic blood pressure. C. Change in mean arterial pressure. D. Change in heart rate. SS, salt sensitivity; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; HR, heart rate.

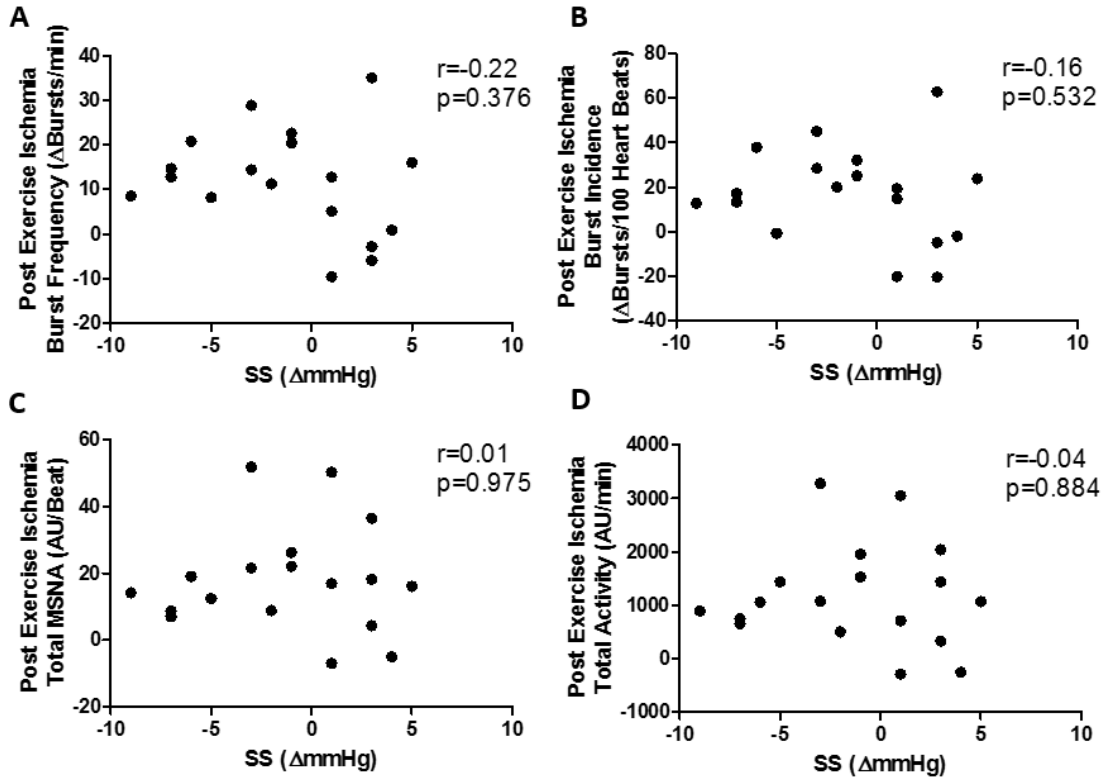

Figure 2.7 **Muscle Sympathetic Nervous Activity Response to Post Exercise Ischemia.** The increase in sympathetic activity was not found to be related to salt sensitivity. A. Change in burst frequency. B. Change in burst incidence. C. Change in total MSNA. D. Change in total Activity. SS, salt sensitivity; MSNA, muscle sympathetic nervous activity; AU, arbitrary units.

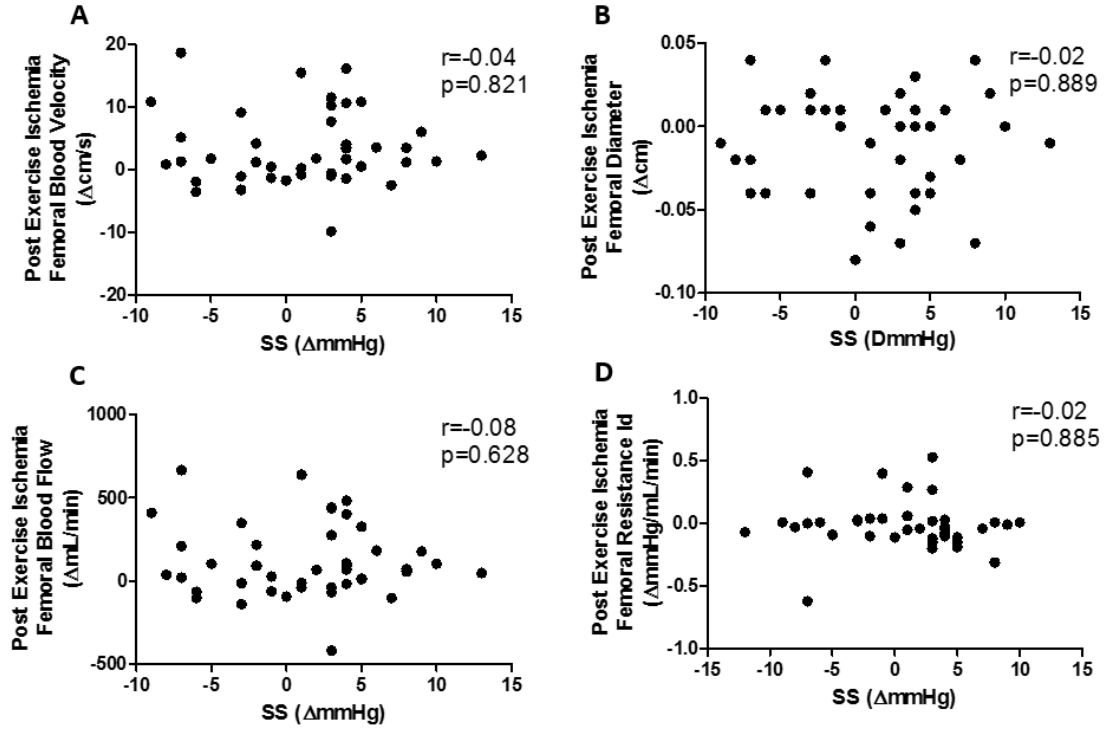

Figure 2.8 **Common Femoral Artery Responses to Post Exercise ischemia.** No relationship exists between salt sensitivity and any measurement made from the femoral artery during post exercise ischemia. A. Change in femoral artery blood velocity. B. Change in femoral artery diameter. C. Change in femoral artery blood flow. D. Change in femoral artery resistance index. SS, salt sensitivity; contraction; Id, index.

2.3.2.3 Venous Distension

The veins of the lower arm were distended by bolus infusion of isotonic saline during circulatory occlusion of the limb. Venous distension resulted in an increase in BP, HR, MSNA, and femoral blood flow (all $p<0.05$), while femoral blood velocity, diameter, and resistance index were not increased from baseline (both $p>0.05$). No significant association was found between SS and the venous distension induced changes in BP (see figure 2.9 A-C), HR (see figure 2.9 D), sympathetic activity (see figure 2.10), or femoral artery measures (see figure 2.11).

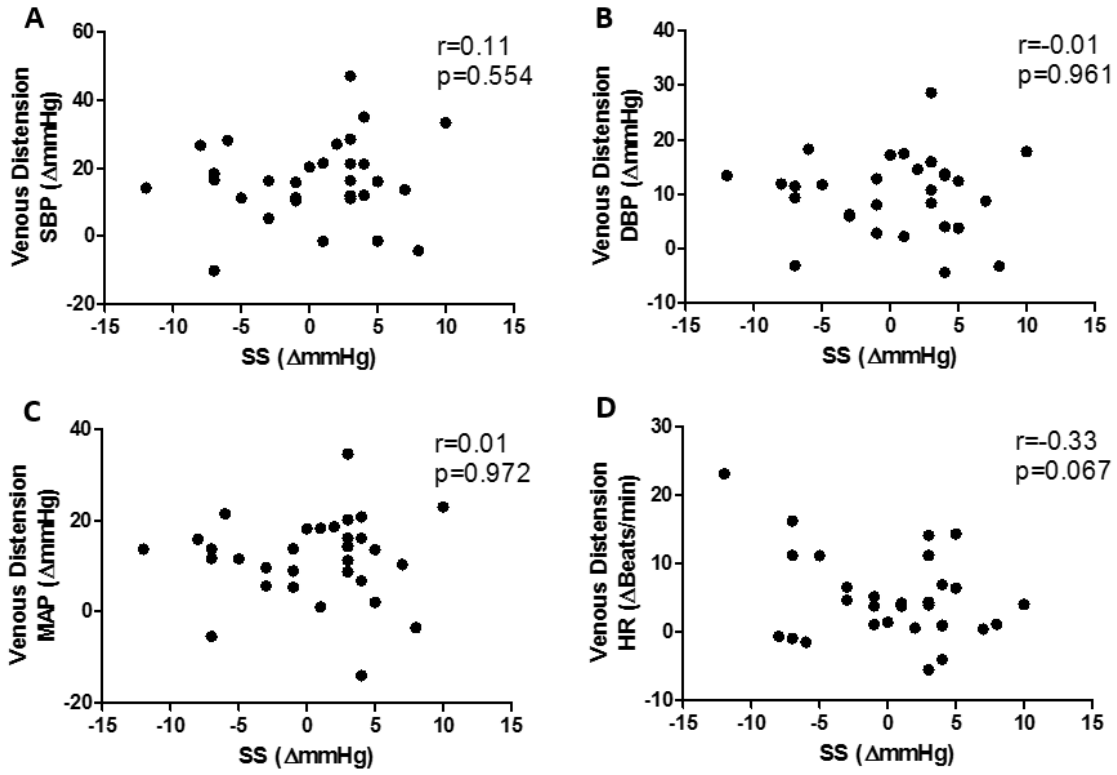

Figure 2.9 **Blood Pressure and Heart Rate Responses to Venous Distension.** No relationship between the increase in systolic, diastolic, mean arterial pressure or heart rate was found with salt sensitivity. A. Change in systolic blood pressure. B. Change in diastolic blood pressure. C. Change in mean arterial pressure. D. Change in heart rate. SS, salt sensitivity; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; HR, heart rate.

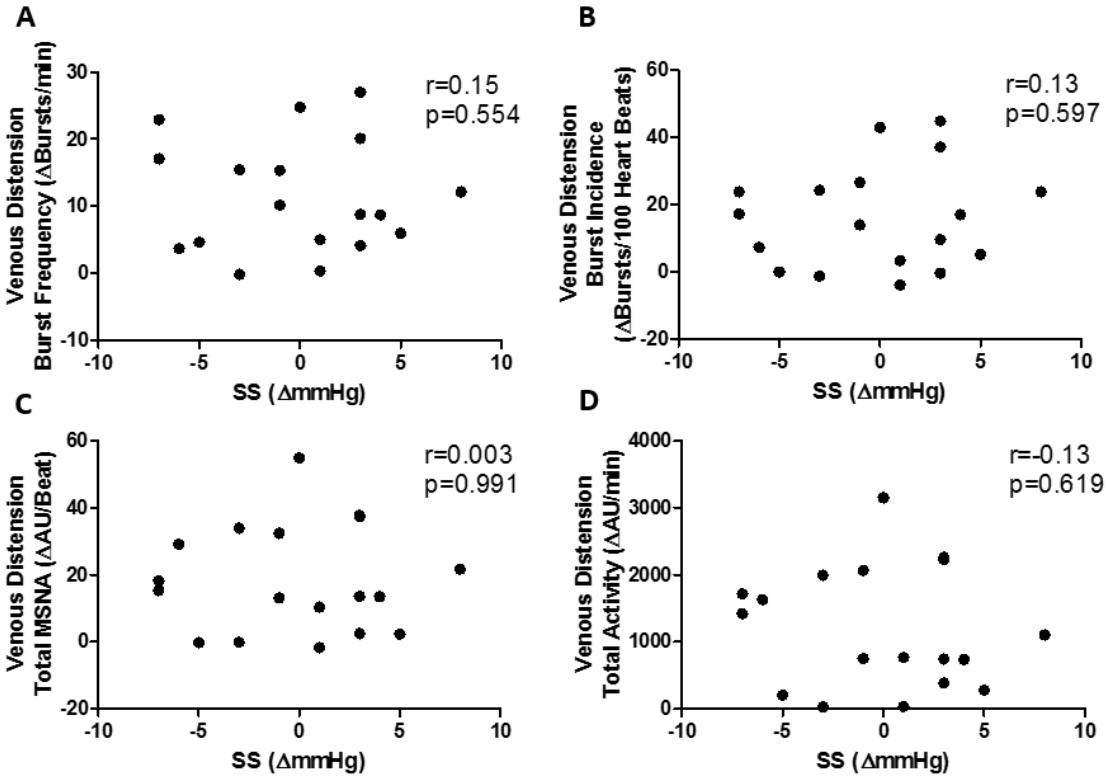

Figure 2.10 **Muscle Sympathetic Nervous Activity Response to Venous Distension.** The increase in sympathetic activity was not found to be related to salt sensitivity. A. Change in burst frequency. B. Change in burst incidence. C. Change in total MSNA. D. Change in total Activity. SS, salt sensitivity; MSNA, muscle sympathetic nervous activity; AU, arbitrary units.

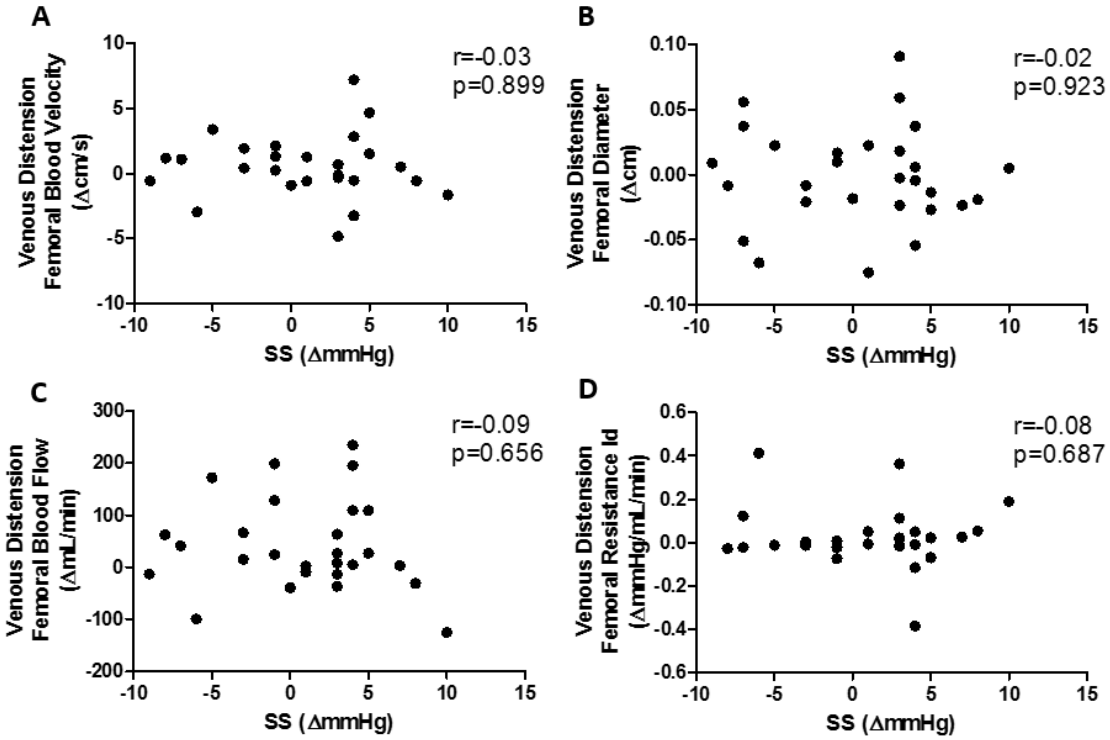

Figure 2.11 **Common Femoral Artery Responses to Venous Distension.** No relationship exists between salt sensitivity and any measurement made from the femoral artery during venous distension. A. Change in femoral artery blood velocity. B. Change in femoral artery diameter. C. Change in femoral artery blood flow. D. Change in femoral artery resistance index. SS, salt sensitivity; contraction; Id, index.

2.3.2.4 Cold Pressor Test

The cold pressor test was performed by submerging the dominant hand of the participant into ice water for two minutes. The cold pressor test caused in an increase in BP, HR, MSNA, and femoral resistance index (all $p<0.05$), while femoral blood velocity, blood flow, and diameter were not increased from baseline (both $p>0.05$). No significant association was found between SS and the venous distension induced changes in BP (see figures 2.12 A-C), HR (see figure 2.12 D), sympathetic activity (see figure 2.13), or femoral artery measures (see figure 2.14).

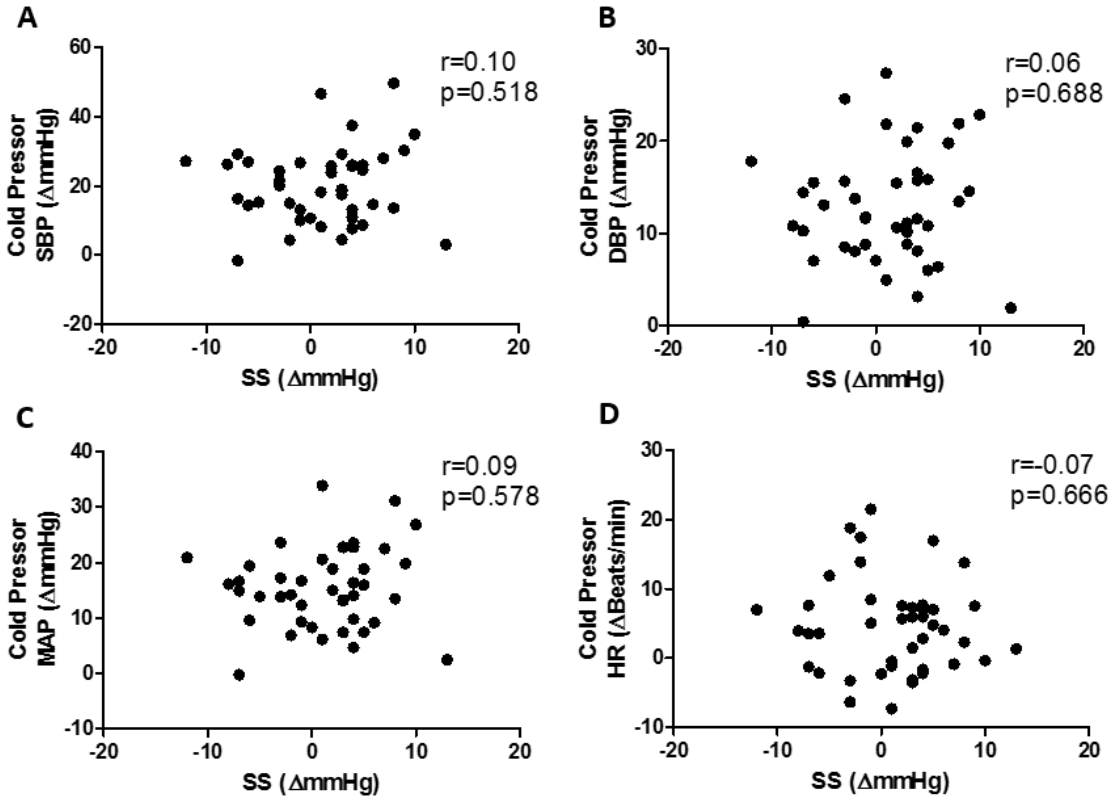

Figure 2.12 **Blood Pressure and Heart Rate Responses to the Cold Pressor Test.** No relationship between the increase in systolic, diastolic, mean arterial pressure or heart rate was found with salt sensitivity. A. Change in systolic blood pressure. B. Change in diastolic blood pressure. C. Change in mean arterial pressure. D. Change in heart rate. SS, salt sensitivity; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; HR, heart rate.

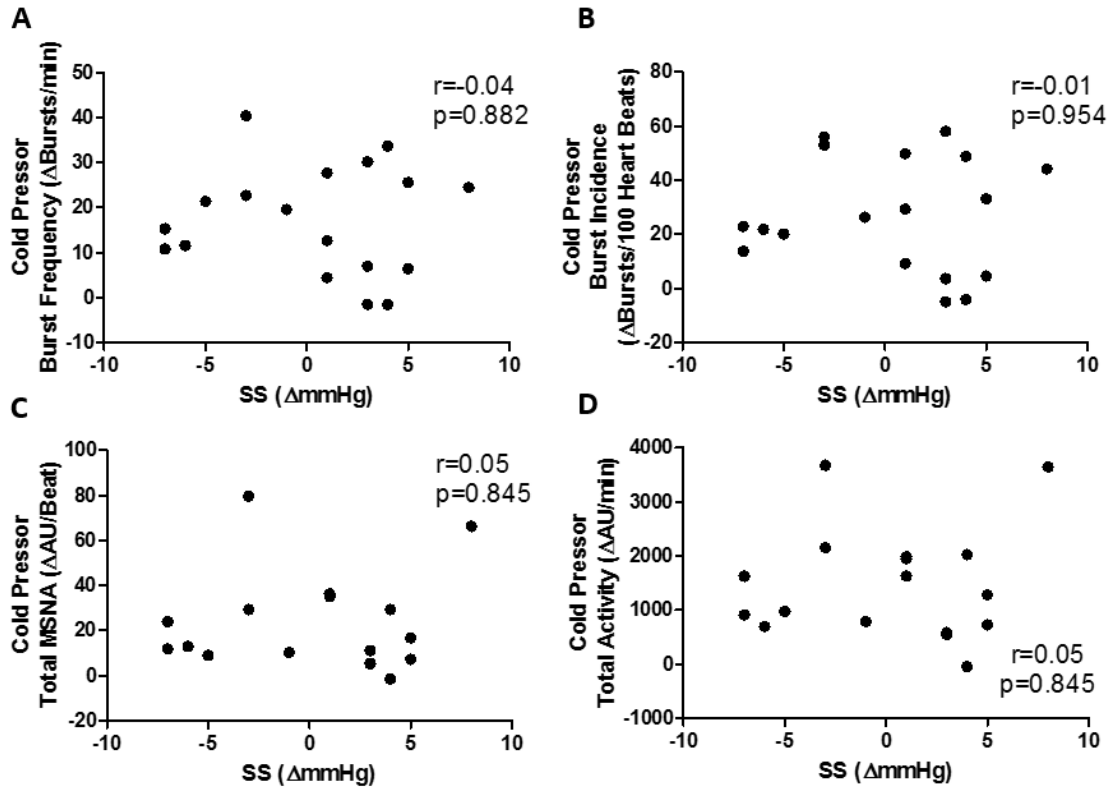

Figure 2.13 **Muscle Sympathetic Nervous Activity Response to the Cold Pressor Test.** The increase in sympathetic activity was not found to be related to salt sensitivity. A. Change in burst frequency. B. Change in burst incidence. C. Change in total MSNA. D. Change in total Activity. SS, salt sensitivity; MSNA, muscle sympathetic nervous activity; AU, arbitrary units.

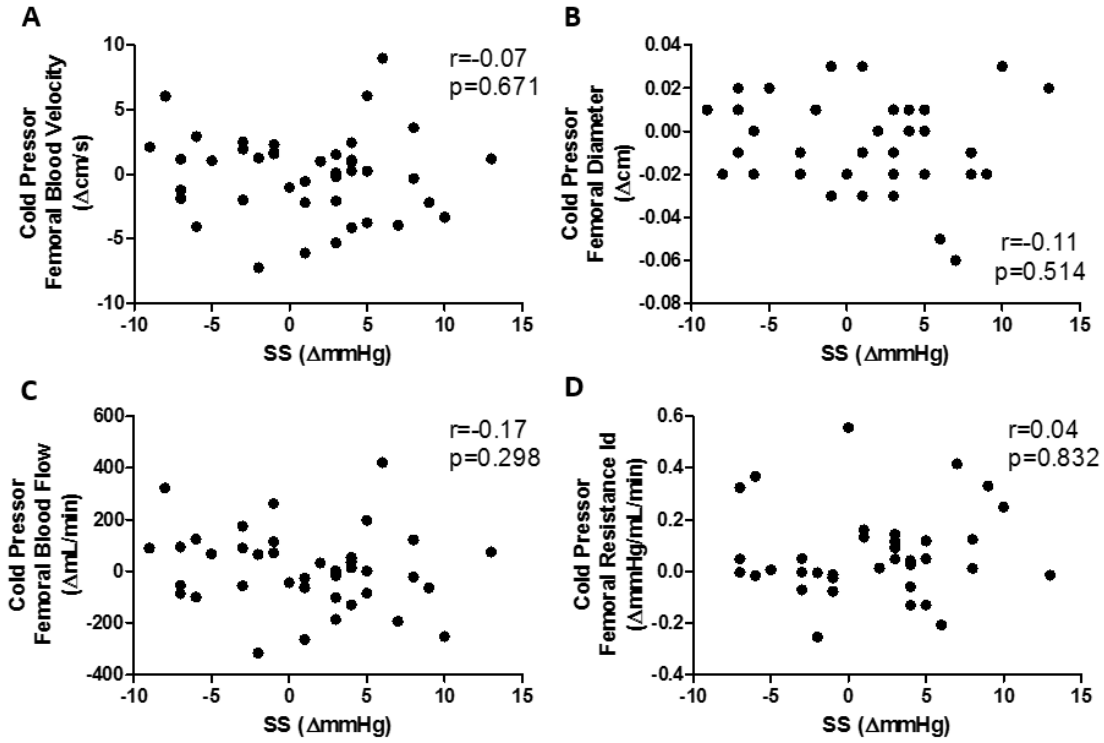

Figure 2.14 **Common Femoral Artery Responses to the Cold Pressor Test.** No relationship exists between salt sensitivity and any measurement made from the femoral artery during the cold pressor test. A. Change in femoral artery blood velocity. B. Change in femoral artery diameter. C. Change in femoral artery blood flow. D. Change in femoral artery resistance index. SS, salt sensitivity; contraction; Id, index.

2.4 Discussion

2.4.1 Primary Findings

To our knowledge, this is the first investigation to examine the relationship between SS and neurocirculatory responses to tests of peripheral reflex stimulation in non-hypertensive humans. BP, MSNA, and femoral artery resistance index responses were not greater in adults with higher SS for any test performed. Therefore, our results are contrary to the hypothesis that SS status would be directly related to reflex sympathoexcitation. Despite this, several novel findings resulted from this analysis. These include a direct relationship of SS with baseline MSNA. Additionally, SS was inversely related to the handgrip responses of HR, MSNA, and femoral artery resistance index. There was also a direct relationship between SS and the handgrip response of femoral artery blood flow. These results suggest that central integration of sympathoexcitatory peripheral reflexes are not different in adults when examined relative to SS status.

Few studies have been done examining neurovascular reactivity in the context of SS. Kenney et al. (64) used anesthetized non-hypertensive DSS and DSR rats fed a normal sodium diet to test responses to direct sciatic nerve stimulation. They found exaggerated BP responses in the DSS rat suggesting that phenotypic differences exist between DSS and DSR rats for BP reactivity. In normotensive adults habitually consuming sodium, mental stress elicited exaggerated responses of BP and heart rate variability in those with elevated SS (13, 28, 29, 129). Campese et al. (15) examined hypertensive adults consuming low and high sodium diets after 5, 10, 15 and 20

minutes of standing, and 40 minutes of ambulation. They found a greater increase in plasma norepinephrine in those with elevated SS during both standing and ambulation suggesting hyper-responsiveness of the sympathetic nervous system. Our results are not consistent with these previous studies, and may be due to differences in species or stressors used.

However, not all available literature has found exaggerations in BP and/or sympathetic responsiveness to stressors in adults with elevated SS. Omvik et al. (97) examined hypertensive adults habitually consuming sodium during bicycle exercise and did not find an increase in BP reactivity in those with elevated SS. When considering all available research including the current results, there does not appear to be a global augmentation of reflex reactivity in those with high degrees of SS. This would suggest that the common elements (i.e. central discharge of sympathetic activity, efferent nervous activity, and sympathetic transduction) of these responses are unlikely responsible for SS related alterations in reactivity found by some researchers (13, 15, 28, 29, 129). Therefore, if differences exist in neurovascular responsiveness in non-hypertensive adults with elevated SS, the alterations in function would likely reside in higher brain centers and/or afferent nervous activity.

Regardless of reactivity, the current analysis found that SS status was related to baseline MSNA. Although it was not possible to separate the effects of SS from those of age this is still a potentially important finding. Interestingly, Miyajima and Yamada (83) studied normotensive Japanese adult males grouped according to their SS status and examined resting MSNA during a high (≈271 mmol/day) and low (≈68 mmol/day) sodium diet. The lower SS group exhibited a decrease in resting MSNA on the high sodium diet, while the group with higher SS did not. Therefore, it is possible that the

higher resting MSNA associated with elevated SS in the current study is due to differences in sympathetic suppression during a habitually high sodium intake (group mean sodium excretion 165.5±9.0 mmol/24hr; see table 2.1). Like others (132), we did find a positive relationship between SS and age which may confound our findings. However, when Miyajima and Yamada (83) found reduced sympathetic inhibition on a high sodium diet in adults with elevated SS, the age range was restricted 19-25 years suggesting this effect is independent of age. Although the current study was not powered to detect differences in subgroups, limiting the sample to men under 30 years of age with successful nerve recordings (n=9) still displays a prominent trend for greater resting MSNA with higher SS status (MSNA total activity r=0.613, p=0.070). The potential of SS status to influence resting sympathetic activity is important because high resting sympathetic activity is linked to the development of essential hypertension (117). Additionally, high resting sympathetic activity has been found in adults with high normal BP (52), which is associated with elevated risk for future cardiovascular disease (125). Indeed, elevations in SS are associated with the development of future hypertension (8, 86, 120), and decreased survival on long term follow-up (130). Therefore, it is reasonable to suspect that the greater development of hypertension in non-hypertensive adults with elevated SS may be due to elevations in resting sympathetic activity.

2.4.2 Isometric Handgrip Exercise

Although the pressor response to isometric handgrip was not increased with regards to SS, there appears to be differences in the mechanism for BP support during muscle contraction. An inverse relationship between MSNA response and SS exists, suggesting that those with the lowest SS display a handgrip induced increase in BP

that is largely driven by sympathetic activity and vascular resistance, indexed by femoral artery resistance.

2.4.3 Limitations

Although the current analysis made several important and novel findings, there are limitations to the study design. One significant limitation of the current study is that participants were tested during their habitual sodium intake, but not during controlled high and low sodium conditions. Recent evidence suggests that high dietary sodium causes augmented RVLM reactivity in salt resistant rat models (3). It is possible that a similar relationship would be further augmented in a highly SS rat or human model, and habitual sodium intake is not high enough, or is too variable to uncover such a finding. The current study provides an important first pass examining the effects of SS status on sympathoexcitation. Additionally, the use of habitual sodium allows for the greatest generalizability of findings to daily life. However, future research should investigate the effect of controlled sodium consumption on sympathoexcitatory responses relative to SS. The somewhat narrow range of SS values studied does represent a limitation as a range containing higher values may display different relationships to the current study. However, as mentioned in the methods, expressing SS in this data set by other BP measures (e.g. office pressure) increased the range of values found without changing the primary conclusions of the study. Another limitation of the current study is a lack of direct manipulation of the central nervous system. Invasive procedures are necessary to directly experiment on the key areas of the brain stem related to sympathetic activity. However, for obvious ethical reasons this could not be done on healthy human participants. Future animal

based research is needed to explore specific brain regions to confirm that there are no SS induced differences in key sympathetic nervous system brain areas.

2.4.4 Conclusions

The results of the current study suggest that there is not a global over reactivity of the sympathetic nervous system in healthy non-hypertensive adults with elevated salt sensitivity of blood pressure in the range of SS examined. This suggests that there are not differences brought on by salt sensitivity status in the discharge, efferent conduction, or transduction of sympathetic responses to stimulation. However, baseline muscle sympathetic activity was related to salt sensitivity and may contribute to the future development of hypertension. Additionally, the support of blood pressure reactivity to static handgrip exercise is different depending on salt sensitivity status, and warrants future research.

Chapter 3

SYMPATHETIC BAROREFLEX SENSITIVITY IS NOT RELATED TO SALT SENSITIVITY STATUS IN NON-HYPERTENSIVE ADULTS

3.1 Introduction

The central nervous system is critical to the control of blood pressure and therefore blood flow throughout the body (104). The baroreflex constantly modulate the autonomic nervous system to maintain blood pressure around a set operating point. Baroreceptors in the carotid artery and aorta sense BP induced mechanical stretch and send excitatory signals via the glossopharyngeal nerve and vagus nerve, respectively. These signals are received by the nucleus tractus solitarius (NTS), which sends excitatory signals to the caudal ventrolateral medulla (CVLM) (122). The CVLM then sends an inhibitory signals to decrease the tonic activity of the rostral ventrolateral medulla (RVLM) resulting in decreased sympathetic activity and BP. This reflex continually functions to modulate BP at rest and during exercise. In fact, baroreflex sensitivity is increased during isolation of the metaboreflex component of the exercise pressor reflex (24, 44, 56, 57, 60). Baroreflex sensitivity refers to the ability to maintain blood pressure near the blood pressure operating point.

Baroreflex sensitivity is low in many disease states (27, 41, 89, 124), and is thought to carry clinical implications for the development of cardiovascular disease (110). Rodent models exhibiting genetically elevated salt sensitivity (SS) display impaired cardiac (11, 35, 88, 90, 108) and sympathetic (39, 54, 82) baroreflex sensitivity. Importantly this impairment of baroreflex sensitivity occurs prior to the development of hypertension, and is thought to contribute to its development (35, 39). Therefore, the examination of baroreflex function in humans relative to SS could provide important insights into neurovascular regulation at rest and during

sympathoexcitation prior to the onset of classical cardiovascular risk factors like hypertension.

SS status of normotensive adults (8, 120) and children as young as six years old (86) predicts the future development of hypertension; yet the baroreflex function of non-hypertensive humans have never been evaluated relative to SS status. Additionally, baroreflex function during reflex sympathoexcitation has never been studied in this context. Therefore, the aim of this analysis is to determine baroreflex sensitivity relative to SS status under habitual sodium consumption at rest and during isolated metaboreflex activation. We hypothesize SS status will be inversely related to baroreflex sensitivity meaning those with the greatest SS will have impaired baroreflex function during habitual sodium consumption.

3.2 Methods

3.2.1 Participants and Visit Schedule

All procedures and protocols utilized are in adherence to the Declaration of Helsinki of 1975, as revised in 1983, and were approved by the Institutional Review Board (IRB) of the University of Delaware. This analysis represents a part of a larger study. Therefore, the methods and participants described are the same as those described previously (see chapter 2) with the exception of methods specifically related to the analysis of baroreflex function. The methods described in this chapter are abbreviated as they are more fully described in chapter 2. Prior to the data collection visit participants underwent two screening visits separated by a SS assessment. Participants were recruited from the Newark, DE, USA community, and are representative of the local community.

3.2.1.1 Screening Visits

Participants underwent two screening visits with separate IRB approved consents. The first visit was performed at the Nurse Managed Health Center at the University of Delaware prior to participation in the SS assessment protocol. During the initial screening visit standard health history forms and health screening tests were performed: medical history questionnaire, physical activity readiness questionnaire, resting blood pressure, resting 12-lead electrocardiogram, height measurement, weight measurement, and blood sample. The purpose of this initial screening visit was simply to exclude participants with chronic conditions from participating in the SS assessment. Participants were also excluded if they were not between 22-60 years old, or used hormone replacement therapy, tobacco, or nicotine products. The second screening visit and informed consent were performed after the SS assessment, and took place at the University of Delaware Cardiovascular Research Laboratory. The second screening visit was used to confirm that there were no major changes in health status or medication/drug usage. During this visit participant characteristic measurements were performed: resting blood pressure, resting electrocardiogram, height, and weight.

3.2.1.2 Salt Sensitivity Assessment

SS was assessed via a 21 day controlled feeding study consisting of a 7 day run-in period (100 mmol Na+/d) followed by a two phase randomized crossover 7-day diet perturbation: low sodium (LS); 20 mmol Na+/d and high sodium (HS); 300 mmol Na+/d. On the final day of the HS and LS phases 24hr urine collections were collected and analyzed for sodium excretion to ensure diet compliance. Twenty-four hour BP was assessed on the final day of the LS and HS phase (Model 90207;

Spacelabs Medical, Issaquah, WA, USA). SS was calculated as the change in 24hr mean arterial BP (MAP), 24hr MAP HS – 24hr MAP LS.

3.2.1.3 Measurements Prior to Data Collection Visit

Participants performed a 3 day diet record and 24hr urine collection to assess habitual sodium intake. The 24hr urine collection was used to assess urine volume, osmolality (Advanced 3D3 Osmometer; Advanced Instruments, Norwood, Massachusetts, USA), free water clearance, and fractional sodium, chloride and potassium excretion (Easy Electrolyte Analyzer; Medica, Bedford, Massachusetts, USA). The 3 day diet record and 24hr urine collection produced similar results for sodium intake and excretion, respectively. To decrease redundancy, the 3 day diet record results are not reported for this aim. Participants reported to the laboratory for the data collection visit after avoiding alcohol, caffeine, and exercise for 24hrs, and fasting for 4 hours.

3.2.2 Experimental Measurements

During the data collection, participants laid in a recumbent position with head and left leg supported slightly above the plane of the body. The non-dominant hand was kept level with the heart. The dominant arm was kept at approximately a 30° horizontal angle from the body. Beat-by-beat arterial BP was measured from the middle finger of the non-dominant hand using a servo-controlled finger photoplethysmographer calibrated to the manufacturer's recommendations (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands). Finger pressures were level corrected post hoc to match the baseline finger pressure to baseline brachial artery pressure (Dinamap Dash 2000; GE Medical Systems,

Milwaukee, WI, USA) as done previously (87). Heart rate (HR) was determined via electrocardiograph lead II tracing (Dinamap Dash 2000; GE Medical Systems, Milwaukee, WI, USA). Respiratory movements were monitored using a thoracic strain-gauge pneumograph to insure normal breathing patterns free of Valsalva maneuvers.

Muscle sympathetic nervous activity (MSNA) was recorded using microneurography (121). A primary tungsten recording microelectrode was inserted in the peroneal nerve behind the fibular head. A reference microelectrode was inserted 2-3 cm from the primary microelectrode. The nerve signal was amplified (factor = 70,000), bandpass filtered (700-2,000 Hz), rectified, and integrated (time constant 0.1 s) using a nerve traffic analyzer (Nerve Traffic Analyzer, model 662c-3; University of Iowa Bioengineering, Iowa City, IA, USA). Criterion used to confirm that the nerve signal obtained was MSNA rather than skin sympathetic nervous activity include: absence of afferent activity during light stroking of the skin, increased efferent activity during voluntary end-expiratory apnea, and spontaneous cardiac cycle gaiting of efferent activity. The MSNA signal was analyzed offline using a custom LabVIEW program (32) which provides synchronized beat-by-beat data of MSNA, BP, and HR. The program identifies MSNA bursts by an R-wave gating approach. The mean value of the three largest bursts were assigned a value of 100 arbitrary units (AU); and all other bursts were scaled accordingly. Baseline MSNA values are reported as burst frequency (burst per unit of time normalized to 1 minute, bursts/min), burst incidence (bursts/ 100 heart beats), total MSNA (burst height [AU]/heart beat) and total activity (burst frequency*mean burst height, AU/min).

3.2.3 Experimental Protocol

Baseline baroreflex sensitivity was assessed during a five minute rest period. Immediately after the rest period two minutes of static handgrip exercise at 40% of maximal voluntary contraction was performed. Results of the handgrip trial were part of another analysis with distinctly different hypotheses (see chapter 2). Approximately 5s prior to the cessation of muscle contraction, an occlusion cuff placed around the upper arm was inflated to 240 mmHg (Rapid Cuff Inflator; Hokanson, Bellevue, Washington, USA). Arterial occlusion continued after muscle contraction (i.e. post exercise ischemia; PEI) for 3 minutes and 15s trapping the metabolites created during contraction. The first 15s of PEI was excluded from analysis in an attempt to analyze baroreflex function during a relatively steady state. The entirety of the remaining 3 minutes was used to examine baroreflex function.

3.2.4 Data Analysis

The arterial baroreflex control of MSNA during rest (5 minutes), and PEI (3 minutes), was assessed as previously done in our laboratory (44). This technique quantifies the baroreflex sensitivity around the operating point by evaluating the slope of the relationship between spontaneously occurring variations in diastolic BP and MSNA (see figure 3.1) (56, 57, 60, 95, 121). This method provides similar baroreflex slopes to that of the modified Oxford approach (i.e. an invasive approach) (50). Diastolic BP was grouped into 1, 2, and 3 mmHg pressure bins throughout the time segments of interest. However, results were similar regardless of bin size. Therefore to reduce redundancy, only the 2 mmHg bin data is shown and discussed. Burst incidence per bin (bursts count in a BP bin/heart beats in the same bin; bursts/100 heart beats) as well as total MSNA per bin (total height of all MSNA burst in a BP

bin/heart beats in the same bin, AU/beat) were used to correlate MSNA over diastolic BP. All data was weighted to account for the number of cardiac cycles within each bin (44, 95). Bins without MSNA activity were included in the analysis. A minimum of r=0.5 was used as an inclusion criterion (44, 96). The slope of the linear regression line represents the arterial baroreflex sensitivity of each participant. In addition to the sympathetic baroreflex sensitivity assessment, a cardiac baroreflex assessment was also made during the rest period. Cardiac baroreflex sensitivity was calculated using the sequencing method with a minimum acceptable r value of 0.8. The R to R interval and systolic blood pressure were regressed for each sequence of four or more consecutive cardiac cycles moving up or down in a parallel fashion. The average slopes of all individual regression lines going up and then all regression lines going down were calculated as an indices of baroreceptor sensitivity. This analysis was performed using HemoLab software. All continuous measurements were recorded at 1,000 Hz with the PowerLab data acquisition system and analyzed with LabChart 7 software (ADInstruments, Colorado Springs, Colorado, USA), or a custom designed LabVIEW program (32).

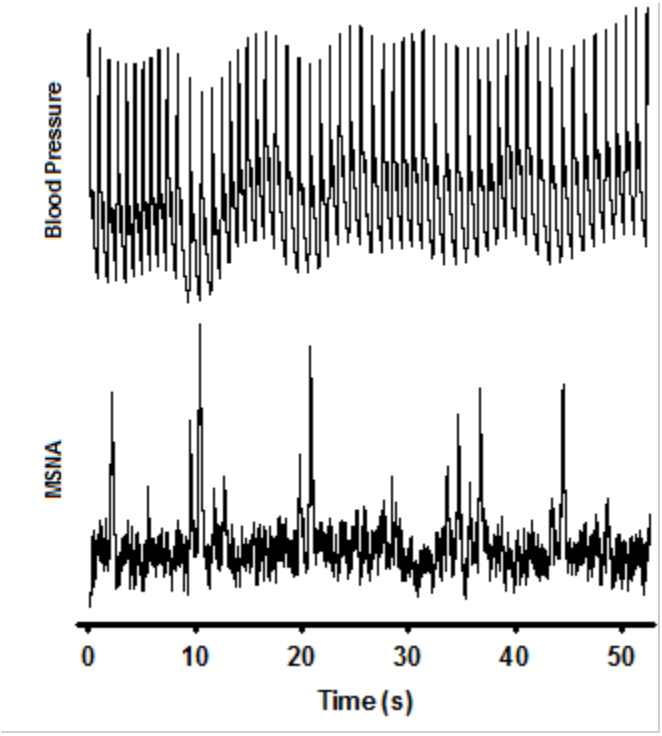

Figure 3.1 **Example of Spontaneously Occurring Oscillations in Blood Pressure and Muscle Sympathetic Activity.** This original tracing clearly depicts the peaks and valleys that occur during steady state blood pressure (top tracing), and the increase in sympathetic activity associated with each diastolic blood pressure valley (bottom tracing).

3.2.5 Statistical Analysis

Linear regression analysis was used to examine variables relative to SS, or other measurement variables. Paired t-tests were used to determine differences between resting and PEI measurements. All variables regressed over SS were also tested using multiple regression to confirm relationships independent of age. Additionally, exploratory analysis was performed examining men only and women only for all linear regression analyses. Results were similar between sexes suggesting the findings of the group as a whole were not affected by examining men and women

together. Therefore, sex specific results are not shown in the results section. Exploratory analysis was also performed using various BP measures to assess SS to be sure the primary conclusions were not affected by the limited range found using 24 hour mean arterial pressure (MAP). This secondary analysis did not affect the primary conclusions. Consequently, it is not shown in the results section. Alpha level of significance was set at $p<0.05$ for all statistical tests. Values expressed as mean ± SEM.

3.3 Results

3.3.1 Participants

Fifty participants were recruited for the current analysis, and underwent SS assessment. Two participants withdrew, and two participants were excluded based on poor proof of increased sodium consumption during the HS diet vs the LS diet (<100Δmmol/24hr). Adequate MSNA records were not found in 27 participants due to a lack of 3:1 signal to noise ratio or lack of relationship between sympathetic activity and diastolic BP at rest (minimum r=0.50). The data in the current analysis represents the data collected from the remaining 19 participants. Participant characteristics are shown in Table 3.1. SS was related to age (r=0.48; p=0.038), and screening HR (r=0.56; p=0.012). No relationship was found between SS and any other screening or baseline biochemical parameter.

Table 3.1: Participant Characteristics

Screening Demographic Data	Mean ± SEM	SS r Value	SS p Value
N (Sex Coded: M1,F2)	19 (13/6)	0.03	0.912
Race (Coded: W1, B2) #	W15, B3, O1	0.22	0.376
Age (Years)	36 ± 3*	0.48	0.038
Height (cm)	174 ± 2	-0.17	0.498
Mass (kg)	75.4 ± 3.0	-0.04	0.870
BMI (kg/m^2)	24.7 ± 0.7	0.09	0.715
SBP (mmHg)	115 ± 2	-0.01	0.958
DBP (mmHg)	69 ± 2	0.01	0.980
MAP (mmHg)	84 ± 2	-0.002	0.994
Heart rate (Beats/min)	57 ± 2*	0.56	0.012
Baseline Biochemical Parameters			
Hemoglobin (g/dL)	13.7 ± 0.3	0.07	0.805
Hematocrit (%)	41 ± 1	0.05	0.853
Serum Sodium (mmol/L)	138.1 ± 0.4	-0.08	0.766
Serum Potassium (mmol/L)	4.07 ± 0.08	-0.05	0.852
Serum Chloride (mmol/L)	104.2 ± 0.7	-014	0.595
Plasma OsM (mOsm/kg H_2O)	287 ± 1	-0.19	0.484
Urine Sodium (mmol/24 hr)	156.2 ± 8.2	0.16	0.538
Urine Potassium (mmol/24 hr)	75.8 ± 3.9	-0.38	0.113
Urine Chloride (mmol/24 hr)	198.2 ± 6.6	0.14	0.575
Urine OsM (mOsm/kg H_2O)	904.5 ± 27.9	0.16	0.519
Urine Flow Rate (mL/min)	1.48 ± 0.08	0.28	0.240
Free Water Clearance (mL/min)	-0.77 ± 0.12	-0.02	0.932

Participant demographic data was collected during the screening visit. Urine was collected over the 24 hours prior to data collection visit. Blood was collected at the data collection visit prior to any trial. SS, salt sensitivity; M, male; F, female; SR, W, white; B, black; O, all other; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; OsM, osmolality. # Participants with races other than white or black were not analyzed due to small group size. * $p<0.05$ relation to salt sensitivity.

3.3.2 Trial Characteristics

Trial characteristics from the data collection visit are displayed in table 3.2. No relationship exists between SS and handgrip maximal voluntary contraction ($r=-0.34$; $p=0.156$) for the exercise leading up to PEI. Baseline MAP was related to SS ($r=0.45$; $p=0.049$), while systolic BP (SBP), diastolic BP (DBP), and HR were not related (all $p>0.05$). Baseline MSNA was previously reported (see chapter 2) and is directly related to SS. This relationship was significant for the MSNA measures of burst frequency ($r=0.47$; $p=0.041$) and burst incidence ($r=0.46$; $p=0.046$). PEI induced a significant increase in BP, HR and MSNA (all $p<0.05$). However, SS was not related to any these variables during PEI (all $p>0.05$).

Table 3.2 Trial Characteristics

Trial Characteristics	Mean ± SEM	SS r Value	SS p Value
Handgrip Trial			
MVC (N)	252 ± 21	-0.34	0.156
Baseline Blood Pressure/HR			
SBP (mmHg)	117 ± 2	0.28	0.244
DBP (mmHg)	75 ± 2	0.30	0.218
MAP (mmHg)	94 ± 2*	0.45	0.049
HR (Beats/min)	59 ± 1	0.05	0.848
Baseline Sympathetic Nervous Activity			
Burst Frequency (Bursts/min)	20.2 ± 2.1*	0.47	0.041
Burst In (Bursts/100 Heart Beats)	34.2 ± 3.7*	0.46	0.046
Total MSNA (AU/Beat)	16.0 ± 1.7	0.44	0.058
Total Activity (AU/min)	950.1 ± 100.8	0.43	0.065
PEI Blood Pressure/HR			
SBP (mmHg)	143 ± 4†	0.04	0.883
DBP (mmHg)	90 ± 2†	-0.18	0.471
MAP (mmHg)	112 ± 3†	0.04	0.887
HR (Beats/min)	65 ± 3†	-0.37	0.127
PEI Sympathetic Nervous Activity			
Burst Frequency (Bursts/min)	33.2 ± 2.1†	0.09	0.709
Burst In (Bursts/100 Heart Beats)	53.3 ± 3.9†	0.23	0.363
Total MSNA (AU/Beat)	37.6 ± 3.9†	0.20	0.425
Total Activity (AU/min)	2340.0 ± 230.1†	0.11	0.665

Baseline characteristics represent measurements recorded immediately prior to the handgrip trial used to induce post exercise ischemia. HR, heart rate; SS, salt sensitivity; MVC, maximal voluntary contraction; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; Burst In, burst incidence; Total MSNA, total muscle sympathetic nervous activity, AU, arbitrary units; PEI, post exercise ischemia. * $p<0.05$ relation to salt sensitivity. † $p<0.05$ vs. baseline.

3.3.3 Baroreflex

The results from the sympathetic baroreflex assessment are displayed in table 3.3. No relationship exists between SS and sympathetic baroreflex sensitivity at rest or during PEI (all $p>0.05$; for individual data see figure 3.2). Additionally, no relationship exists between SS and any parameter related to the sympathetic baroreflex assessment (all $p>0.05$). An individual example ($n=1$) of the difference between sympathetic baroreflex function during rest and PEI is presented in figure 3.3. Sympathetic baroreflex sensitivity was not different between baseline and PEI for burst incidence ($p=0.410$), but was increased from baseline to PEI for total MSNA ($p=0.037$; see figure 3.4). Cardiac baroreflex sensitivity at rest for up sequences trended towards lower sensitivity in adults with the highest SS ($r=-0.309$; $p=0.096$), however when utilizing multiple regression to adjust for age this trend was lost ($p=0.712$). Cardiac baroreflex sensitivity at rest for down sequences was not related to SS status ($r=-0.160$; $p=0.398$).

Table 3.3 Baroreflex Characteristics

Trial Characteristics	Mean ± SEM	SS r Value	SS p Value
Baseline			
Mean DBP (mmHg)	75 ± 2	0.30	0.218
DBP Range (mmHg)	17 ± 1	0.27	0.265
DBP Bins	9 ± 1	0.24	0.314
Burst In, Sensitivity (Bursts/100 Heart Beats/mmHg)	-4.76 ± 0.56	0.30	0.207
Total MSNA, Sensitivity (AU/Heart Beat/mmHg)	-2.71 ± 0.32	0.28	0.237
Burst In vs. DBP, r	-0.824 ± 0.035	0.14	0.563
Total MSNA vs. DBP, r	-0.815 ± 0.035	0.11	0.664
Post Exercise Ischemia			
Mean DBP (mmHg)	90 ± 2†	-0.18	0.471
DBP Range (mmHg)	19 ± 1	0.06	0.827
DBP Bins	10 ± 1	0.04	0.862
Burst In, Sensitivity (Bursts/100 Heart Beats/mmHg)	-4.32 ± 0.41	0.08	0.786
Total MSNA, Sensitivity (AU/Heart Beat/mmHg)	-3.78 ± 0.46†	0.03	0.927
Burst In vs. DBP, r	-0.788 ± 0.036	-0.02	0.941
Total MSNA vs. DBP, r	-0.792 ± 0.035	-0.13	0.638

Baroreflex analysis characteristics at rest, and during post exercise ischemia. Values represent analysis utilizing 2 mmHg diastolic blood pressure binning. Results were similar with 1 and 3 mmHg diastolic blood pressure binning. SS, salt sensitivity; DBP, diastolic blood pressure; Burst In, burst incidence; Total MSNA, total muscle sympathetic nervous activity, AU, arbitrary units. * $p<0.05$ relation to salt sensitivity. † $p<0.05$ vs. baseline.

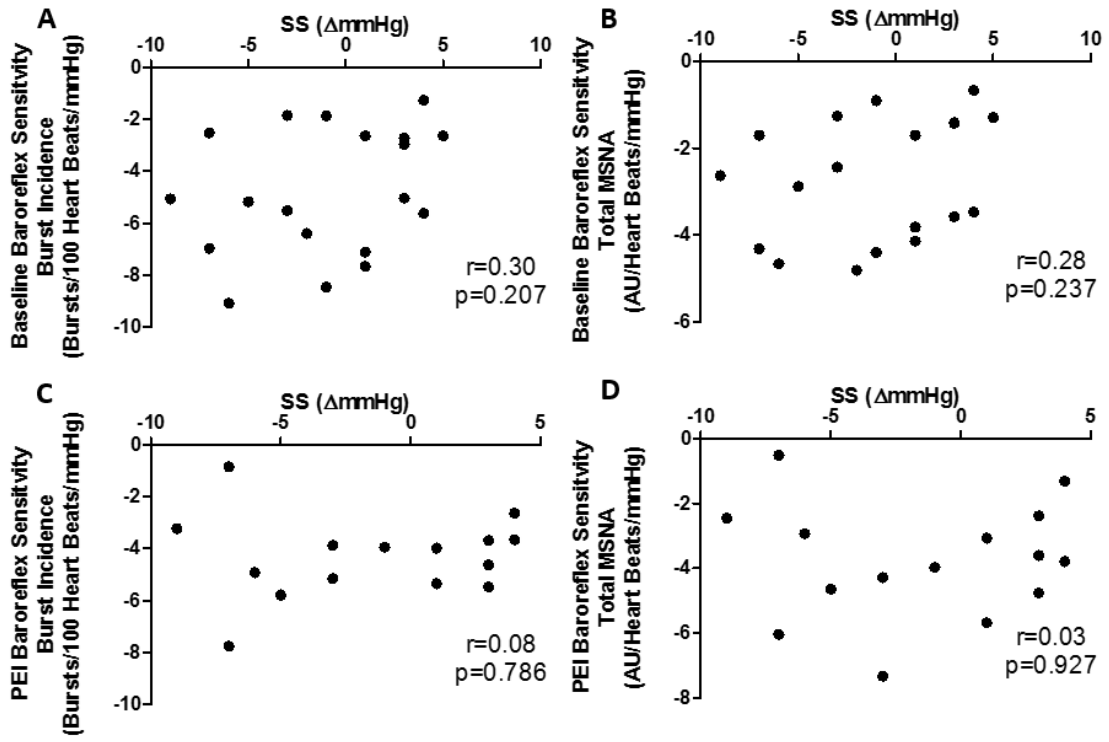

Figure 3.2 **Baroreflex Sensitivity during Rest and Post Exercise Ischemia Relative to Salt Sensitivity.** No relationship exists between baroreflex sensitivity and salt sensitivity. A. Baseline muscle sympathetic nervous activity burst incidence/diastolic blood pressure baroreflex sensitivity. B. Baseline muscle sympathetic nervous activity total/diastolic blood pressure baroreflex sensitivity. C. Post exercise ischemia muscle sympathetic nervous activity burst incidence/diastolic blood pressure baroreflex sensitivity. D. Post exercise ischemia muscle sympathetic nervous activity total/diastolic blood pressure baroreflex sensitivity. SS, salt sensitivity; AU, arbitrary units.

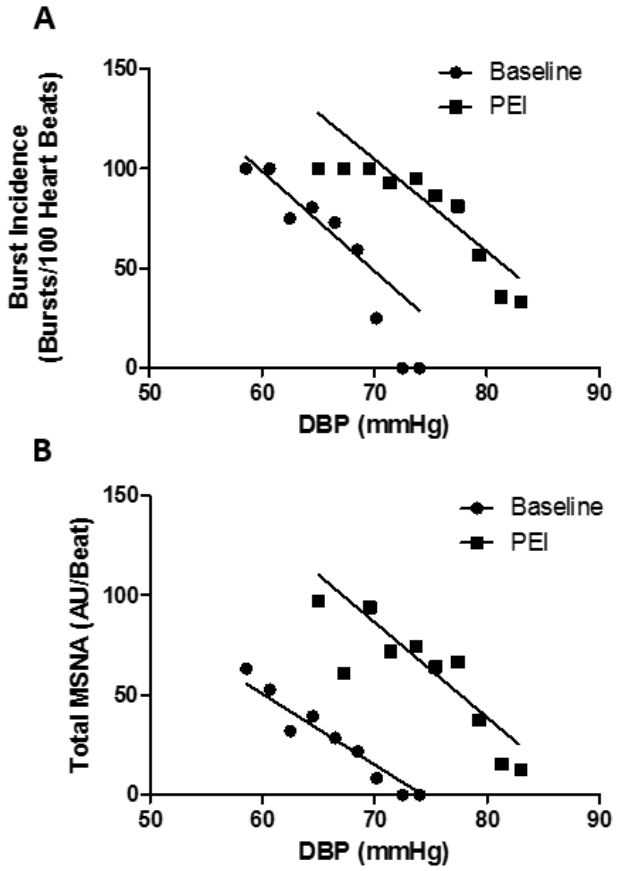

Figure 3.3 **Example Plot of the Relationship between Sympathetic Activity and Diastolic Blood Pressure from One Participant.** Figure shows the increase in diastolic blood pressure during post exercise ischemia compared to baseline. Also evident is the post exercise ischemia induced increase in slope of the regression line (i.e. baroreflex sensitivity) for total muscle sympathetic nervous activity over diastolic blood pressure. A. Muscle sympathetic nervous activity measured as burst incidence regressed over diastolic blood pressure during baseline (r=0.836) and post exercise ischemia (r=0.870). B. Muscle sympathetic nervous activity measured as total activity regressed over diastolic blood pressure during baseline (r=0.886) and post exercise ischemia (r=0.854).

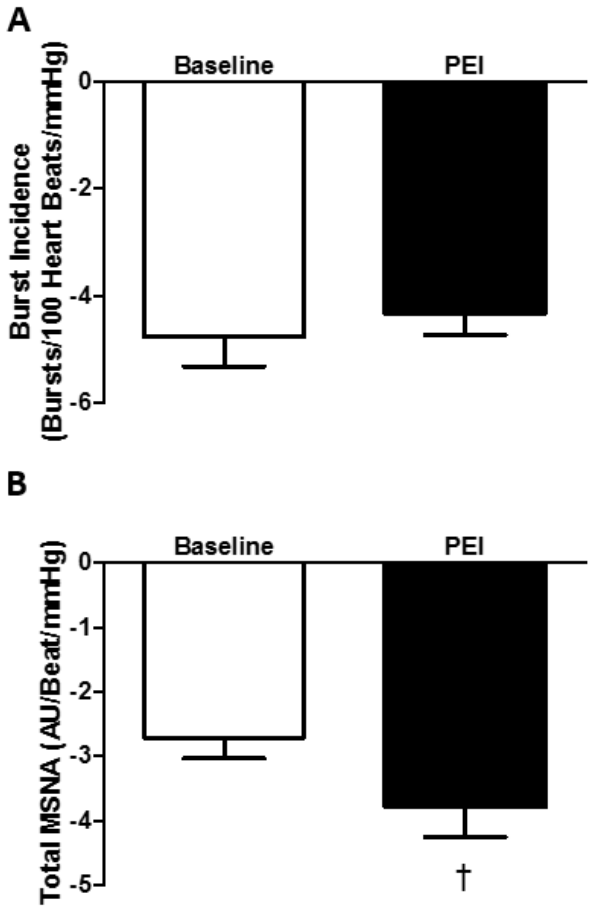

Figure **3.4** **Baroreflex Sensitivity during Rest and Metaboreflex Stimulation.** Baroreflex sensitivity as calculated from MSNA burst incidence does not change during post exercise ischemia compared to baseline. Baroreflex sensitivity as calculated from total MSNA increases during post exercise ischemia compared to baseline. A. Baroreflex sensitivity of muscle sympathetic nervous activity measured as burst incidence over diastolic blood pressure. B. Baroreflex sensitivity of muscle sympathetic nervous activity measured as total muscle sympathetic nervous activity over diastolic blood pressure. PEI, post exercise ischemia; MSNA, muscle sympathetic nervous activity; AU, arbitrary units. † $p<0.05$ vs. baseline.

3.4 Discussion

3.4.1 Primary Findings

To our knowledge, this is the first investigation to examine the relationship between SS and the baroreflex in non-hypertensive humans. Regression analysis between baroreflex sensitivity and SS was not significant. A similar analysis during metaboreflex stimulation was also not significant. Contrary to our hypothesis, we did not find impaired baroreflex sensitivity in those with the greatest degree of SS. This was done during habitual sodium consumption suggesting baroreflex function during daily life is normal in healthy normotensive adults regardless of SS status. In addition to this key finding, this data shows that metaboreflex stimulation resulted in increased baroreflex sensitivity. Exercise induced increased baroreflex sensitivity (24, 44, 56, 57, 60) is now known to occur in healthy adults. These results do not support the hypothesis that impaired baroreflex function accompanies elevated SS in non-hypertensive adults.

Rodent models created to display phenotypic elevations in SS exhibit impaired cardiac (11, 35, 88, 90, 108) and sympathetic (39, 54, 82) baroreflex sensitivity. Gordon and Mark (38) examined Dahl salt sensitive (DSS; phenotypic high SS) and Dahl salt resistant (DSR; phenotypic low SS) rats consuming LS to remove the confounding variable of hypertension. Electrical stimulation of the afferent aortic depressor nerve displayed equal responses for BP, HR, and sympathetic nervous activity suggesting strain differences in baroreflex function are not caused by differences in central integration or efferent nerve activity. Additionally, aortic arch distensibility was not different between rat strains. However, multifiber afferent aortic baroreceptor discharge was less reactive in the DSS strain following a phenylephrine

induced increase in BP. This decrease in reactivity suggests that impaired baroreflex function in the DSS rat is due to less responsive baroreceptors. Additionally, these studies suggest baroreflex function is depressed in the DSS rat strain regardless of dietary sodium consumption and prior to the onset of hypertension. This has led some researchers to propose impaired baroreflex function as the cause for hypertension development in DSS rats (39, 54). Indeed, sinoaortic denervation will convert Sprague Dawley rats into a salt sensitive strain allowing for dietary sodium to induce hypertension (98). Our results do not show a relationship between baroreflex sensitivity and SS status in non-hypertensive humans. Therefore, the current results do not support the theory that baroreflex impairment causes elevated SS. This discrepancy is likely due to species differences.

To our knowledge there is only one previous study examining sympathetic baroreflex sensitivity relative to SS status in humans. Tinucci et al. (123) studied hypertensive adults during a two level controlled sodium diet and found no differences for baroreflex control of MSNA between individuals grouped according to SS status, regardless of diet. The previous literature on SS and baroreflex control of sympathetic activity in humans is scarce. Additionally, no prior human studies exist examining baroreflex function in the context of SS without the presence of hypertension. The current analysis fills this gap in the literature. Our results are in agreement with those of Tinucci et al. (123), and suggest that sympathetic baroreflex sensitivity is not affected by SS status in either normotensive or hypertensive adults.

In recent years, evidence has suggested a lack in correlation between cardiac and sympathetic baroreflex sensitivity assessments (31). There are several differences between these measures of baroreflex function. The baroreflex control of cardiac

efferents are calculated by regressing R-R interval or HR over SBP, while baroreflex control of sympathetic activity is calculated by regressing MSNA over DBP. Therefore, the cardiac baroreflex calculation uses an end organ response for the dependent variable, while sympathetic baroreflex calculations utilize a direct measure of efferent activity for its dependent variable. Another major difference is that the cardiac baroreflex responds to absolute levels of a stimulus, while the sympathetic baroreflex responds primarily to the direction and magnitude of the change in stimulus (36). The key difference between cardiac and sympathetic baroreflex assessments is that cardiac baroreflex sensitivity is thought to directly relate to parasympathetic activity (101), unlike sympathetic baroreflex sensitivity. Both cardiac efferents and sympathetic efferents are important for the regulation of BP, but they do so differently. Coruzzi et al. (19) studied hypertensive adults and found that regardless of diet, cardiac baroreflex sensitivity and heart rate variability were progressively lower as the level of SS increased. In conjunction with the results of Tinucci et al. (123), this would suggest that hypertensive individuals with high levels of SS have normal baroreflex control over sympathetic activity, but impaired control over parasympathetic activity. This work has not been done in normotensive adults relative to SS status, and represents an important question for future research.

3.4.2 Limitations

Although the current analysis filled an important void in the current literature, there are limitations to this study. We tested participants while they consumed their habitual sodium intake, not during controlled high and low sodium conditions. This makes it difficult to make conclusions on the contribution of the baroreflex to SS, as

sodium was not manipulated. Despite this limitation, studying participants in their habitual state provides relevant information for clinical outcomes, as it better relates to daily life. Future research should investigate the effect of controlled sodium consumption on baroreflex function relative to SS status in non-hypertensive adults. The somewhat narrow range of SS values studied does represent a limitation as a range containing higher values may display different relationships to the current study. However, as mentioned in the methods, expressing SS in this data set by other BP measures (e.g. office pressure) increased the range of values found without changing the primary conclusions of the study. Another limitation is the lack of direct BP manipulation to examine the full range of baroreflex sensitivity. Without direct stimulation it is impossible to know where participant operating points were relative to their individual centering point, threshold boundary, and saturation boundary. Regardless, the primary measurement of baroreflex function is sensitivity, and the spontaneous oscillation approach provides similar baroreflex slopes to that of the modified Oxford approach, an invasive approach utilizing direct BP manipulation (50). Therefore, the primary measurement of baroreflex function would not likely be different if the modified Oxford approach had been utilized.

3.4.3 Conclusions

The results of the current study are that sympathetic baroreflex function is not different regardless of salt sensitivity status, for the range of SS examined, in non-hypertensive adults at rest or during metaboreflex stimulation. This finding suggests that sympathetic baroreflex function is not likely the cause of the heterogeneity of the blood pressure response to dietary sodium, or the increased likelihood of developing hypertension in those with high salt sensitivity. Further research is needed to

determine if cardiac baroreflex sensitivity is affected by salt sensitivity status in non-hypertensive adults.

Chapter 4

CONCLUSION

4.1 Summary

The central goal of this project was to determine the relationship, if any, salt sensitivity (SS) status has with the autonomic nervous system control of blood pressure (BP) during rest and sympathoexcitation. This is important because individuals with high degrees of SS are more likely to develop hypertension (8, 86, 120); a condition thought to be initiated by autonomic nervous system dysfunction (117). Given the known link between hypertension and cardiovascular risk (85), and the increased incidence of hypertension development in individuals with elevated SS (8, 86, 120), it is not surprising that SS status is predictive of cardiovascular related death (130). Therefore, this project represents an important first step to examine the potential link between SS status and the autonomic nervous system as it may contribute to higher cardiovascular mortality in those with high degrees of SS.

The first set of analyses performed for this project examined the blood pressure, muscle sympathetic nervous activity (MSNA), and limb resistance at rest and in response to multiple sympathoexcitatory maneuvers in non-hypertensive adults pre-assessed for SS status. We hypothesized that participants would respond to sympathoexcitation in accordance to their SS status, which would suggest a central nervous system over excitability in adults with high degrees of SS. The results to this analysis did not display a relationship between SS status and BP response in any test performed. Furthermore, no relationship existed between SS status and MSNA or limb resistance responses during post exercise ischemia (i.e. metaboreflex isolation), venous distension, or cold pressor test. Baseline MSNA was directly related to SS

status, which may represent a one potential link between elevated SS and the associated propensity for hypertension development (8, 86, 120). Additionally, differences existed regarding the support for BP during handgrip exercise relative to SS status. Our results suggest that adults with greater SS status increase BP during handgrip primarily with an increase in blood flow not MSNA and resistance.

The current investigation was the first of its kind in normotensive adults, and our results suggest a need for future research. We allowed participants to continue their habitual sodium intake so that findings would have the greatest generalizability to daily life. However, evidence suggests that high dietary sodium causes augmented central nervous system reactivity in salt resistant rats (3). It is possible that such a response would be further augmented in a highly SS individual, and habitual sodium intake is not high enough, or is too variable to uncover such a finding. Therefore, investigating the effect of controlled high and low sodium diets on sympathetic reactivity is one important avenue for future research. Additionally, organ specific blood flow measurements as well as cardiac output measurements during handgrip exercise are needed to further determine how adults with high levels of SS support BP during muscle contraction. This information could prove important to understanding SS related hypertension development if sympathetic activity is indeed the mechanism. With that said, studies using sympatholytic drugs could examine the relationship between resting sympathetic activity and BP relative to SS status.

Our second analysis performed during this project sought to determine sympathetic baroreflex function during rest and sympathoexcitation in non-hypertensive adults pre-assessed for SS status. We hypothesized that adults would display sympathetic baroreflex sensitivity that was inversely related to their SS status.

Such a result would suggest an abnormal baroreflex control of sympathetic activity in adults with high degrees of SS. Our results did not show a relationship between sympathetic baroreflex sensitivity and SS status during rest or metaboreflex isolation. This suggests that sympathetic baroreflex function is normal in non-hypertensive adults regardless of SS status. In doing this analysis we were able to replicate the findings of others that baroreflex sensitivity increases during metaboreflex isolation (24, 44, 56, 57, 60).

Our second analysis was aimed at understanding baroreflex control of MSNA relative to SS status; however, we did not determine cardiac baroreflex sensitivity which relates to the parasympathetic nervous system. Recent evidence suggests a lack in correlation between these important efferent arms of the baroreflex (31). As both cardiac efferents and sympathetic efferents are important for the regulation of BP, it is important to characterize both. Coruzzi et al. (19) studied hypertensive adults and found cardiac baroreflex sensitivity was inversely related to SS status. This work has not been done in normotensive adults relative to SS status, and represents an important question for future research.

4.2 Perspectives

Non-hypertensive adults with elevated salt sensitivity have greater development of hypertension (8, 86, 120), and poorer long term survival compared to adults with lesser degrees of salt sensitivity (130). Determining the cause of salt sensitivity has proved difficult as it is likely multifactorial (69). Emerging evidence from animal literature bolsters the claim that the central nervous system plays an integral part in determining salt sensitivity status (118). Little research has been done linking salt sensitivity status to the central nervous system in humans. Development

of the body of literature linking salt sensitivity to the central nervous system is crucial to understanding the link between salt sensitivity status and cardiovascular disease (84) and death (130), as well as, discovering new avenues for preventing such poor outcomes.

Our findings suggest that resting sympathetic activity, but not sympathetic reactivity or sympathetic baroreflex sensitivity are related to salt sensitivity status. To our knowledge, this is the first evidence in humans to provide a direct link between the sympathetic nervous system and salt sensitivity status in humans. More research is needed to determine the cause and potential effects of these findings.

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APPENDIX

INSTITUTIONAL REVIEW BOARD PROTOCOL APPROVAL LETTER

RESEARCH OFFICE

210 Hullihen Hall
University of Delaware
Newark, Delaware 19716-1551
Ph: 302/831-2136
Fax: 302/831-2828

DATE: June 4, 2013

TO: Evan Matthews, MS
FROM: University of Delaware IRB

STUDY TITLE: [463149-1] Blood Pressure Reactivity in Salt Sensitive Normotensives

SUBMISSION TYPE: New Project

ACTION: APPROVED
APPROVAL DATE: June 4, 2013
EXPIRATION DATE: May 14, 2014
REVIEW TYPE: Full Committee Review

Thank you for your submission of New Project materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office.

Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Jody-Lynn Berg at (302) 831-1119 or jlberg@udel.edu. Please include your study title and reference number in all correspondence with this office.