CENTRAL HEMODYNAMIC RESPONSES TO AN ACUTE SODIUM LOAD

by

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ABSTRACT

Chronically high dietary sodium intake has been associated with elevated blood pressure. Many studies have shown that black adults demonstrate a higher degree of sodium sensitivity and vascular reactivity than white adults. **PURPOSE**: To examine the acute effects of sodium on blood pressure, cardiac output, and peripheral vascular resistance in black and white subjects. We hypothesized that the hypertonic saline infusion would elicit an increase in blood pressure that would be more robust in black subjects. **METHODS**: 11 healthy black and 15 healthy white subjects underwent a hypertonic saline (3%) infusion lasting 60 minutes. Blood pressure and cardiac output were assessed at baseline, 20, 40, and 60 minutes into the infusion. Blood was drawn for the analysis of serum sodium, osmolality, hematocrit, at baseline, 15, 30, 45, and 60 minutes into the infusion. Plasma volume and peripheral vascular resistance were calculated. Results are reported as means ± standard errors and a two way ANOVA was used to compare the effects of time and race. **RESULTS**: During the infusion, serum sodium (135.9 ± 0.4 to 141.7 ± 0.5 mmol/L), osmolality (288.8 ± 0.6 to 297.8 ± 0.7), plasma volume (18.0 ± 1.9%), mean arterial blood pressure (80.7 ± 2.3 to 91.9 ± 2.6 mmHg) and cardiac output (4.3 ± 0.2 to 4.8 ± 0.3 L/min) significantly increased in both groups ($p < 0.05$). There was a time effect for peripheral vascular resistance. White subjects demonstrated a greater increase in plasma volume compared to black subjects (21.46 v 13.78, $p=0.03$). C**ONCLUSION**: The hypertonic saline infusion caused a significant increase in blood pressure that was not different between black and white subjects.

Chapter 1

INTRODUCTION

Many studies have established a link between high dietary sodium intake and elevated blood pressure[1-4]. However, there remains a high degree of inter-individual variability in the blood pressure response to changes in sodium intake[5]. Individuals that exhibit a large change in blood pressure in response to changes in sodium intake are considered to be salt sensitive. While this is not found in all individuals, black adults have a higher degree of salt sensitivity than white adults[4,6]. This is important since sodium sensitivity may predict future hypertension in normotensive adults[7] and also because black adults have a significantly higher prevalence and earlier onset of hypertension than white adults[8].

Whether caused by chronic or acute conditions, blood pressure changes must be due to changes in cardiac output, peripheral vascular resistance, or both. Many studies have found differences in vascular reactivity between black and white individuals. In response to laboratory stressors, black adults demonstrate a higher degree of vasoconstriction and a lesser degree of vasodilation suggesting that black individuals have a more sensitive α-adrenergic receptor[9-11]. This may possibly

contribute to the greater increase in blood pressure in response to increases in sodium intake found in this population.

While most studies have looked at the chronic effect of sodium intake, few have looked at the effect of acute changes in serum sodium on blood pressure. The purpose of this study was to examine the hemodynamic responses to an acute sodium load in black and white subjects. This was accomplished through a 60-minute infusion of a 3% hypertonic saline while blood pressure was recorded. We hypothesized that acute changes in serum sodium would cause an increase in blood pressure in all subjects, but that the change in blood pressure would be greater in black as compared with white subjects. In order to identify the mechanisms underlying changes in blood pressure, cardiac output and peripheral vascular resistance were measured. We hypothesized that the increase in blood pressure would be due to an increase in peripheral vascular resistance, and that increase would be more robust in black subjects.

Chapter 2

RESEARCH DESIGN AND METHODS

***Subjects**:* Twenty-six subjects (15 men and 11 women) reported to the Human Performance Lab for a verbal and written explanation of the procedure by the principal investigator. Before proceeding with the screening, an informed consent approved by the University of Delaware Human Subjects Review Board was signed by all subjects. Healthy subjects (men and women) between the ages of 18 and 44 were recruited from the local area. Only subjects with no signs or symptoms of diseases, normal blood work, normal resting ECG, and normal blood pressure were included in the study. Exclusion criteria for the PAR-Q included affirmative answers to questions 1-4 and 9, and not more than one affirmative answer to questions 5-8. Subjects were also excluded if they were obese, hypertensive, used tobacco, or took prescription medications. Pregnant women were also excluded from the study. Since the menstrual cycle can affect fluid balance, women were tested in the early follicular phase only.

Black and white subjects were specifically recruited for the study. Exclusion criteria based on race included reporting a race other than African American or Caucasian as well as reporting that parents are of two different races.

***Screening Procedures**:* The screening involved a medical history questionnaire, PAR-Q, resting blood pressure, resting 12-lead ECG, body fat assessment using the skin fold technique, along with height and weight measurement for body mass index calculation. A blood sample was taken and sent to Christiana Care Hospital in Delaware for a complete blood count, a lipid profile (i.e., total cholesterol, HDL-C, LDL-C, and triglycerides), fasting glucose, and liver function tests (i.e., aspartate transaminase and alanine transaminase), and kidney function tests (i.e., creatinine and blood urea nitrogen). The blood sample was also analyzed for hematocrit, sodium, potassium, chloride, and osmolality in the Human Performance Lab. All subjects completed a sub-maximal bike ergometer test for assessment of fitness level. The test began with a 2-minute warm up at 20 watts. Resistance was then set to 75 watts and increased 25 watts per minute. During the exercise test, the PARVO Medics Metabolic Cart continuously monitored O_2 consumption; blood pressure, ECG and heart rate were assessed at the end of each 2-minute stage. The test continued until 85% of age-predicted maximum heart rate was achieved.

GENERAL PROCEDURES

***Subject Instructions**:* Subjects were instructed to drink 750 ml of water the day before the experimental day as well as to avoid high sodium foods. Starting 12 hours before their arrival to the Human Performance Lab, subjects avoided strenuous exercise, caffeine, alcohol, or medications. Subjects then fasted over night. The morning of the experimental day subjects ingested another 250 ml of water before arriving at the lab at approximately 7:00 am. All subjects' hydration status was confirmed upon arrival by measuring urine specific gravity with a hand held refractometer (Reichert, Inc. Depew, NY). Subjects were considered sufficiently hydrated if the specific gravity of the urine was below 1.025.

Saline Infusion Protocol: A hypertonic saline solution (3.0%) was infused (Abbott Lifecare 5000 infusion pump, Rockwall, TX) through an intravenous catheter placed in the left arm. The infusion lasted one hour and the total volume was 0.15 ml of saline per kg of body weight per minute.

Blood Pressure, Heart Rate, Respiration: Beat by beat arterial blood pressure was monitored using the Finapres (Finapres Medical Systems, The Netherlands). The Finapres was calibrated to an upper arm cuff. The blood pressure measurements were taken at the finger. Respiratory bands around the chest and abdomen were used to measure breath depth and frequency (Inductotrace System, Ambulatory Monitoring,

Inc., Ardsley, NY). A five-lead ECG was used during the protocol to measure R-R interval and heart rate (Dinamap Dash 2000, GE Medical Systems, Milwaukee, WI). Respiration, blood pressure, and ECG measurements were recorded by Windaq software throughout the infusion (DATAQ Instruments, Akron, OH).

Cardiac Output: Cardiac output was measured using a PARVO Medics metabolic cart (TrueOne 2400 Metabolic Measurement System, ParvoMedics, Sandy, UT) according to methods described by Hyardi et al [12]. At baseline the subject breathed normal room air through a valve while expired air was collected for a measurement of VCO_2. This continued until VCO_2 levels became stable, which was approximately five minutes. The mean VCO_2 for this time was calculated and used to derive cardiac output at all time points. VCO_2 was then reassessed at the end of the protocol. After the VCO_2 measurement, the subject began the CO_2 rebreathing technique. The subject breathed room air for about 30 seconds and then switched to a gas mixture with a higher percent of oxygen and carbon dioxide than room air. The air was re-breathed for 15 to 25 seconds while the oxygen and carbon dioxide content of exhaled air was measured. The rebreathing technique was repeated 3 to 5 times each time cardiac output is measured. Cardiac output was measured at baseline, 20, 40, and 60 minutes into the infusion and all successful trials were averaged and reported in liters per minute.

Blood Draws: An intravenous catheter was placed in the right arm for blood samples. Blood draws occurred at baseline, 15, 30, 45, and 60 minutes into the infusion. Blood was drawn for the analysis of hematocrit, sodium, potassium, chloride, and osmolality at all time points. The total blood drawn through out the protocol was 150 ml.

Blood Analysis: Blood samples were processed within the lab immediately after the infusion. Hematocrit was measured by filling three capillary tubes with whole blood, centrifuging the tubes for 5 minutes, and reading the scale next to the tubes (Readacrit Centrifuge, Becton Dickinson and Company, Parsippany, NJ). The average from the three tubes was used. Plasma volume was calculated from hematocrit according to Greenleaf et al[13]. Sodium, potassium, and chloride levels were analyzed in triplicate from serum using the Easy Electrolytes Na/K/Cl Analyzer (Medica, Bedford, MA). Osmolality was measured from serum using the Advanced Osmometer (Advanced Instruments, Inc. Norwood, MA). Osmolality was assessed using the freezing-point depression method. Standard calibration and quality control procedures were followed for all blood analyzing instrumentation.

DATA ANALYSIS

ECG was peak detected using the Windaq software to derive both R-R interval and heart rate (Windaq waveform browser, Advanced CODAS software). Blood pressure

measured by the Finometer was peak and valley detected in order to derive beat by beat diastolic and systolic blood pressure. A representative 5 minutes of data were compared at baseline, 20, 40, and 60 minutes.

STATISTICAL ANALYSIS

Justification for sample size: G Power software was used to calculate sample size. We hypothesized that we would find a difference in mean arterial blood pressure of 10 mmHg between groups. Using a standard deviation of 7 mmHg from recent literature[14], G Power calculated sample size, with a power of 0.80 and significance at $P < 0.05$, to be 14 subjects per group.

Values are reported as mean ± standard error. A two-way repeated measures ANOVA was used to assess the differences between time points and groups during the infusion (SPSS 12.0). Linear regression analysis (least squares method, SigmaPlot 8.0) was used to examine the serum sodium-MAP relationship, the osmolality-MAP relationship, and the hematocrit- MAP relationship. A single line was applied to all of the data (e.g. top panel, Figure 3), and then applied to the data on an individual basis (middle panel, Figure 3). The slopes of each individual were used as indices of MAP responsiveness to acute changes in serum sodium (bottom panel, Figure 3), plasma osmolality, and plasma volume, respectively. Significance was set at $P < 0.05$.

Chapter 3

RESULTS

Subject characteristics are listed in Table 1. There were no significant differences in mean arterial pressure, systolic blood pressure, height, weight, BMI, body fat percent, hip to waist ratio, or estimated VO_2 max between the two groups. Diastolic blood pressure was significantly higher in black subjects. All subjects had normal baseline clinical blood values. None of the subjects were considered hypertensive according to The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [15].

During the infusion, there was a significant increase in serum sodium and osmolality while hematocrit significantly decreased as shown in Figure 1 ($p < 0.001$). Serum sodium was significantly higher in white subjects ($p = 0.001$) but the change in serum sodium between the two groups was not different. Osmolality and hematocrit were not different between black and white subjects. Plasma volume increased significantly throughout the infusion. White subjects demonstrated a significantly greater increase in plasma volume of 21.46% as compared with black subjects, which demonstrated an increase of 13.78% ($p = 0.034$).

Mean arterial pressure increased significantly ($p < 0.001$) from baseline throughout the infusion as shown in Figure 2. There was a significant time effect of cardiac output ($p < 0.001$) and peripheral vascular resistance ($p = 0.026$) also. There were no significant differences between black and white subjects in any of these variables.

Figure 3 represents the break down of cardiac output for black and white subjects. The top panel shows the heart rate and the bottom panel shows the stroke volume. There was a significant increase in heart rate ($p < 0.001$) and a significant time effect of stroke volume ($p = 0.002$) but there were no differences between the groups for either of these variables.

The top panel of Figure 4 represents the change in serum sodium regressed against the change in mean arterial pressure in black subjects ($r = 0.1412$, $p = 0.4331$) on the right and white subjects ($r = 0.479$, $p < 0.0009$) on the left. The middle panel represents one individual subject's blood pressure response to the acute increase in serum sodium. The bottom panel includes all subjects' individual blood pressure response. The bold line represents the average mean arterial pressure and serum sodium for all subjects. There were no significant differences between the groups in the r-value ($p = 0.769$) or the slope ($p = 0.315$) of this relationship. Mean arterial pressure was also regressed against hematocrit and osmolality. In the relationship between hematocrit and mean arterial pressure there were no differences between groups in the r-value ($p = 0.532$) or slope ($p = 0.545$). In the relationship

between osmolality and mean arterial pressure there were also no differences between groups in the r-value ($p = 0.718$) or slope ($p = 0.409$).

Table 1. Subject characteristics collected at the time of the screening. P value represents the differences between black and white subjects based on an unpaired t-test.

	All Subjects	Black Subjects	White Subjects	P value
N (total)	26	11	15	
N (men)	15	7	8	
N (women)	11	4	7	
Age (years)	25.6 ± 1.3	24.6 ± 2.3	26.3 ± 1.6	0.535
Height (cm)	172.3 ± 2.03	173.9 ± 3.94	171.1 ± 2.09	0.499
Weight (kg)	75.8 ± 3.1	82.2 ± 6.4	71.1 ± 2.2	0.079
BMI (kg/m)	25.1 ± 0.7	26.2 ± 1.3	24.3 ± 0.7	0.178
Hip to Waist Ratio	0.80 ± 0.02	0.83 ± .02	0.78 ± 0.02	0.129
Body Fat (%)	14.95 ± 1.31	15.05 ± 2.03	14.88 ± 1.77	0.951
SBP (mmHg)	114.7 ± 1.9	117.7 ± 2.3	112.5 ± 2.7	0.181
DBP (mmHg)	70.5 ± 1.5	74.1 ± 2.0	67.9 ± 1.8	0.033*
MAP (mmHg)	85.8 ± 1.3	88.6 ± 1.8	83.7 ± 1.8	0.070
Pulse Pressure (mmHg)	44.2 ± 1.6	43.6 ± 2.5	44.6 ± 2.2	0.775

Figure 1. The stimulus: serum sodium (top), osmolality (middle), and hematocrit (bottom) at baseline and throughout the 60-minute infusion in black (closed circles) and white (open circles) subjects. During the infusion there was a significant change in each variable. At each time point there was a significant difference in serum sodium between black and white subjects ($p < 0.05$).

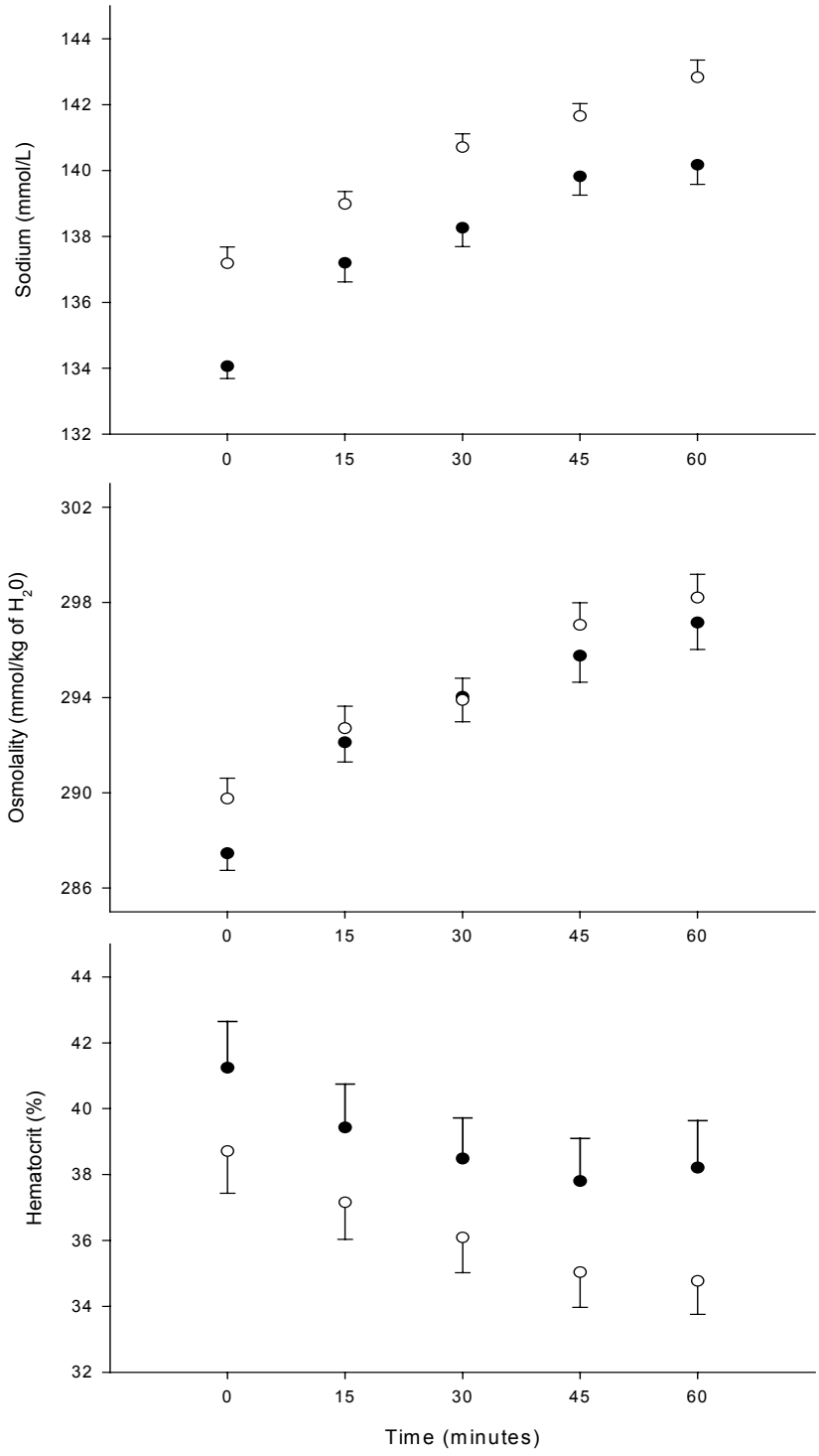

Sodium (mmol/L)
144
142
140
138
136
134
132
0
15
30
45
60
Osmolality (mmol/kg of H_2O)
302
298
294
290
286
0
15
30
45
60
Hematocrit (%)
44
42
40
38
36
34
32
0
15
30
45
60
Time (minutes)

Figure 2. The response: mean arterial pressure, cardiac output, and peripheral vascular resistance at baseline and throughout the 60-minute infusion in black (closed circles) and white (open circles) subjects. During the infusion there was a significant change in each variable. ($p < 0.05$)

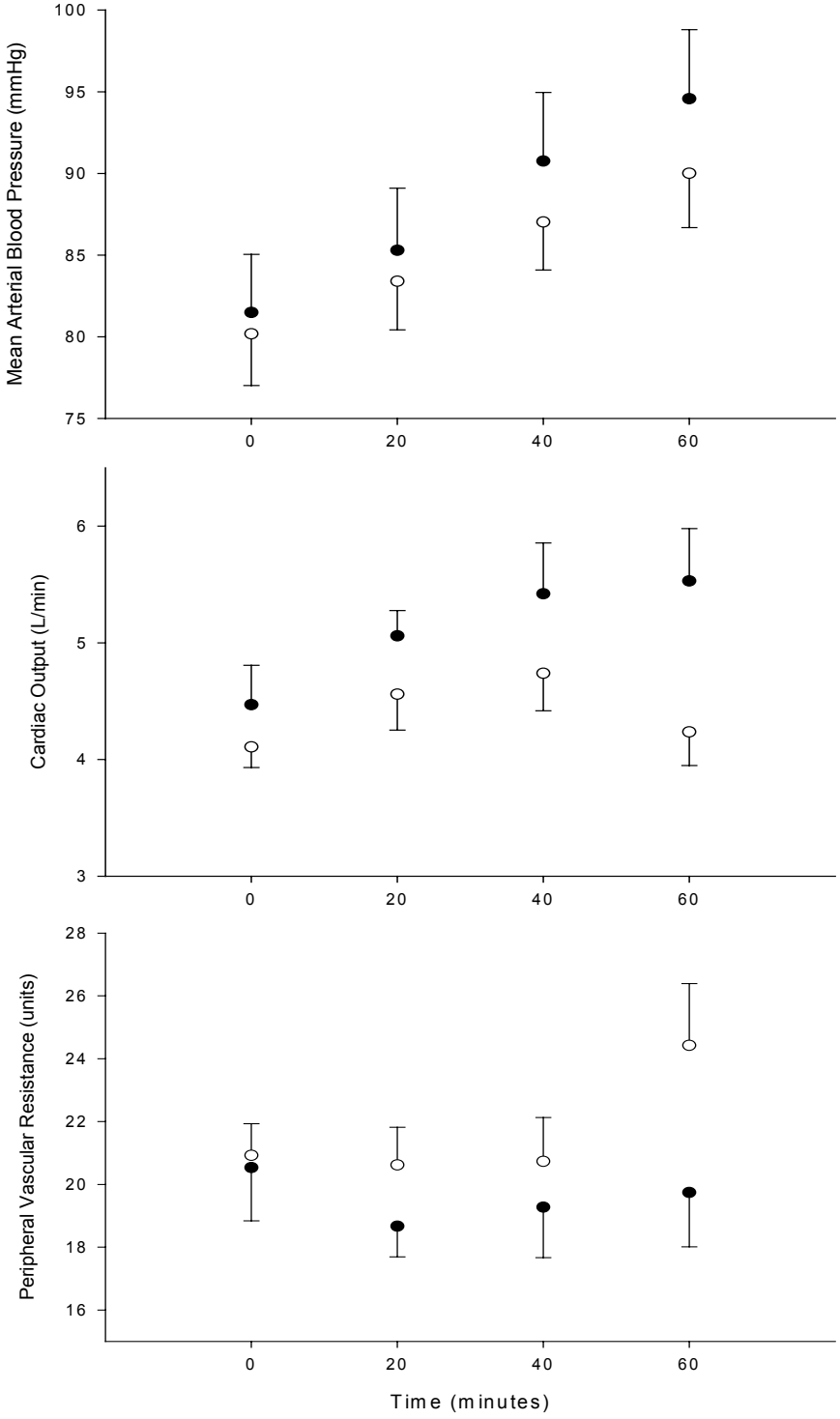
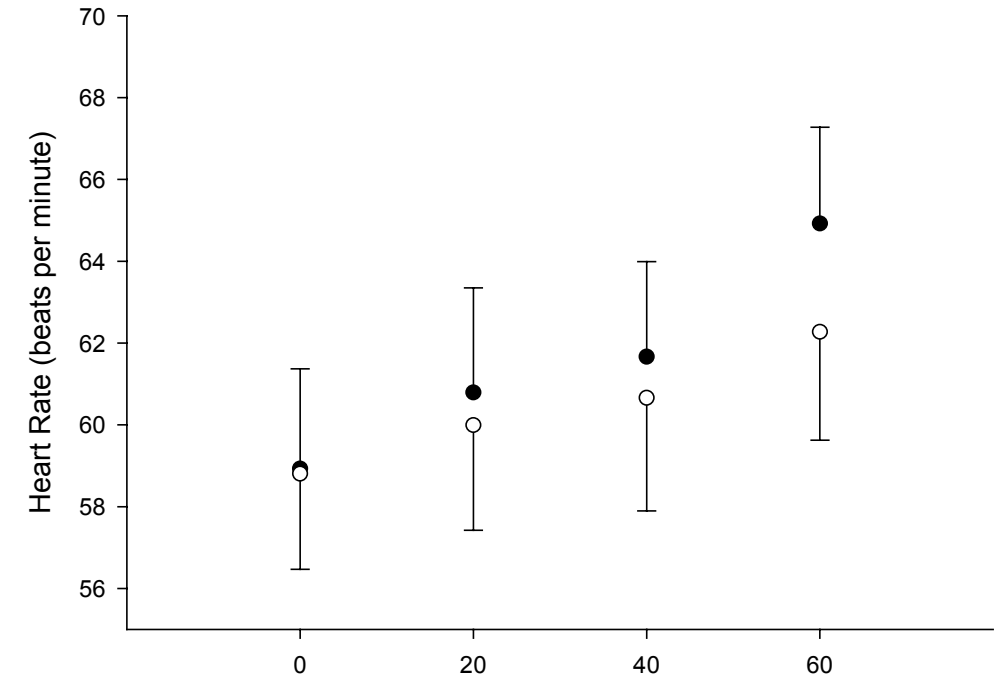

Mean Arterial Blood Pressure (mmHg)
100
95
90
85
80
75
0
20
40
60
Cardiac Output (L/min)
6
5
4
3
0
20
40
60
Peripheral Vascular Resistance (units)
28
26
24
22
20
18
16
0
20
40
60
Time (minutes)

Figure 3. Heart Rate and Stroke Volume. The top graph represents heart rate at baseline and throughout the infusion. The bottom graph represents stroke volume at baseline and throughout the infusion. The open circles represent data from white subjects while the closed circles represent data from black subjects. There was a significant time effect of each variable but no differences between black and white subjects ($p > 0.05$).


Heart Rate (beats per minute)
70
68
66
64
62
60
58
56
0
20
40
60

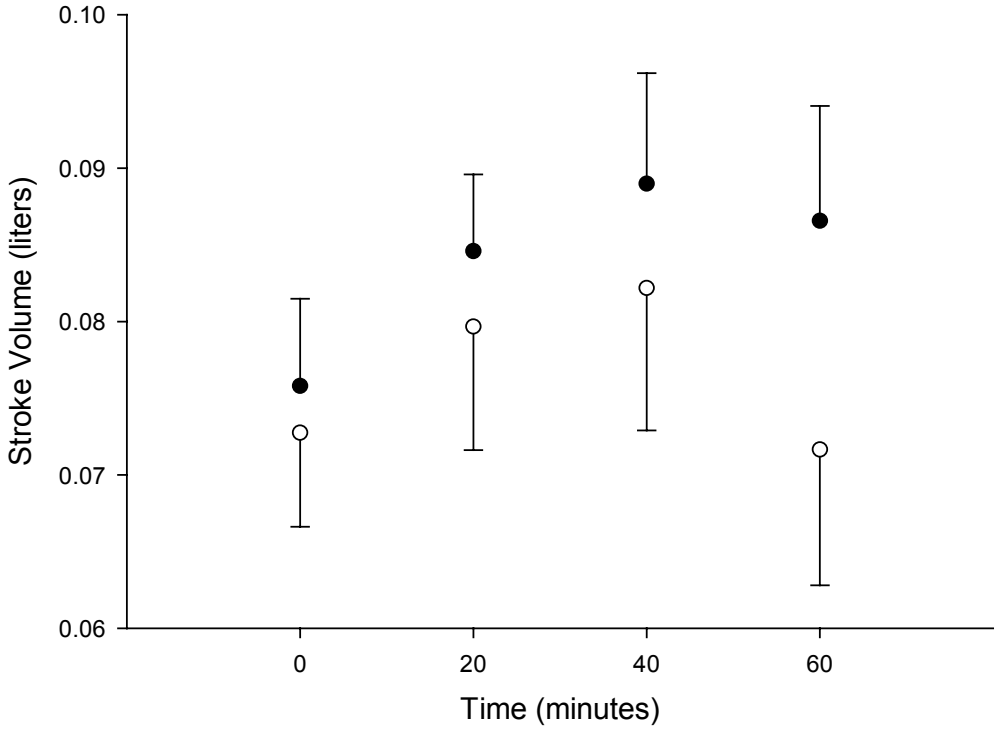
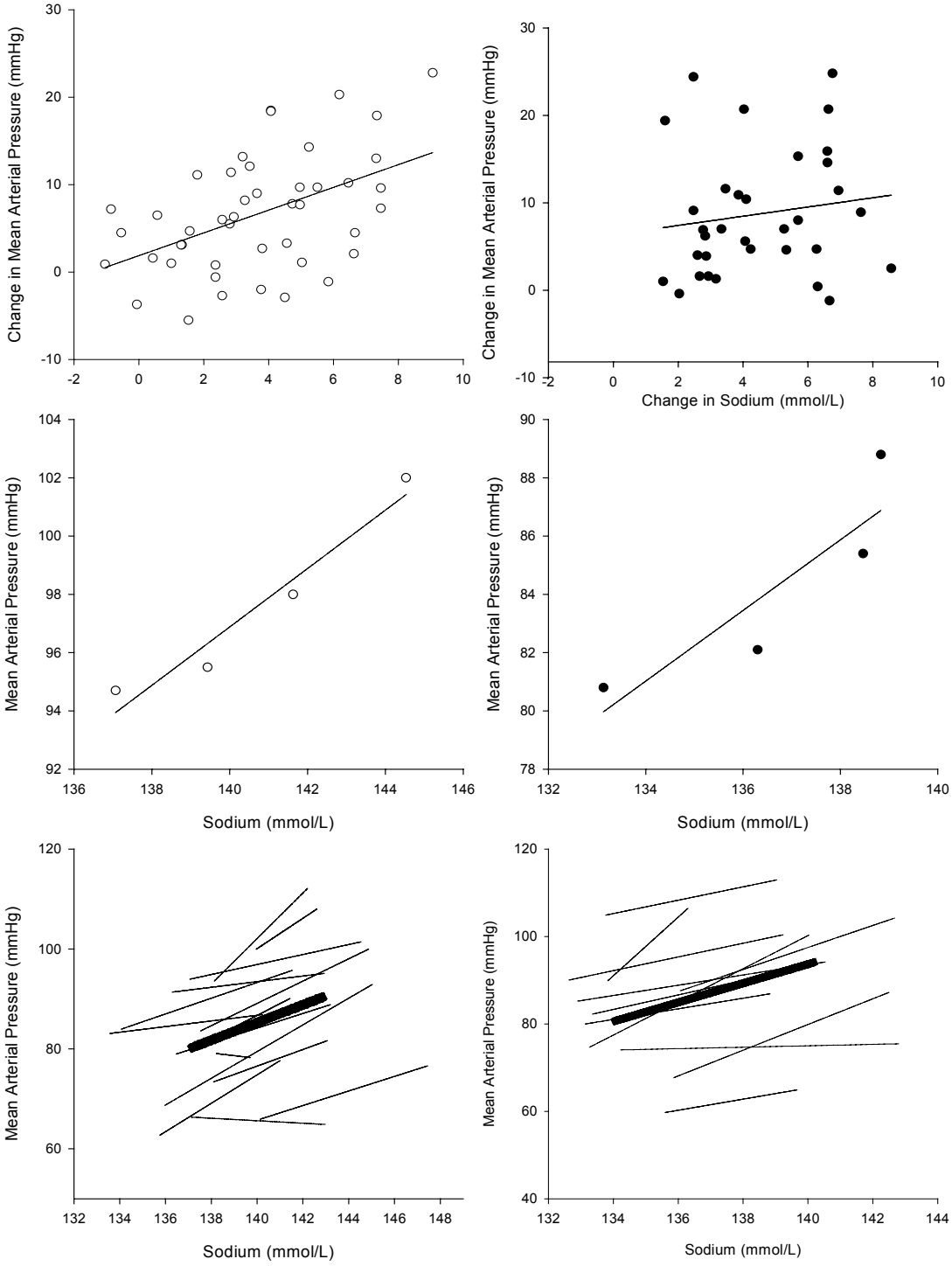

Stroke Volume (liters)
0.10
0.09
0.08
0.07
0.06
0
20
40
60
Time (minutes)

Figure 4. Stimulus-response relationship of serum sodium and mean arterial blood pressure for white (left panel) and black (right panel) subjects. The top graphs represent the change in serum sodium and the change in mean arterial blood pressure for white (n = 15, r = 0.4791) and black (n = 11, r = 0.1412) subjects. The middle graphs represent 1 subject's individual blood pressure response to a change in serum sodium. The bottom graphs represent all subjects' individual responses to changes in serum sodium. The bold lines in each of the bottom graphs represent the mean blood pressure and mean serum sodium for all subjects.


Change in Mean Arterial Pressure (mmHg)
Change in Mean Arterial Pressure (mmHg)
Change in Sodium (mmol/L)
Mean Arterial Pressure (mmHg)
Sodium (mmol/L)
Mean Arterial Pressure (mmHg)
Sodium (mmol/L)
Mean Arterial Pressure (mmHg)
Sodium (mmol/L)
Mean Arterial Pressure (mmHg)
Sodium (mmol/L)

Chapter 4

DISCUSSION

The hypertonic saline infusion was successful in acutely increasing serum sodium, osmolality, and plasma volume. These acute changes caused an increase in blood pressure, and cardiac output, and caused a change in peripheral vascular resistance. The change in blood pressure was not significantly different between the black and white subject groups but the change tended to be greater in black subjects.

A possible reason for the lack in differences between groups in the blood pressure response to the saline infusion may have been a low degree of salt sensitivity in the subjects. Both hypertensive and older adults have a greater prevalence of hypertension than normotensive young adults[7,16]. Since the cohort of subjects used in this study were both young and normotensive, it may not have been possible to see a difference in the blood pressure response between the two groups.

While differences between groups were not found in the relationship of mean arterial pressure and serum sodium, it may still be a useful indicator of salt sensitivity. More research is needed to see whether subjects have similar responses to an acute sodium load, like the one used here, and a chronic manipulation of dietary

sodium intake. If similar results are found in the chronic and acute setting, than this could become a clinical tool in diagnosing salt sensitivity.

Using a retrospective analysis, subjects were classified as normotensive or prehypertensive based on their baseline blood pressure readings taken during their subject screening. Prehypertension is defined as a systolic blood pressure between 120 and 139 mmHg or a diastolic blood pressure between 80 and 89 according to JNC 7[15]. Eight out of a total of 26 subjects were classified as prehypertensive. The slope of the mean arterial pressure and serum sodium relationship in the prehypertensive and normotensive subjects were then compared using an unpaired t-test. Prehypertensive subjects tended to have a higher slope than normotensive subjects (2.67 ± 0.7 vs. 1.51 ± 0.3, $p = 0.077$). This may indicate that in young healthy adults, baseline blood pressure rather than race may better predict blood pressure responses to changes in serum sodium.

Power calculations estimated group size at 14 subjects per group. A limitation of this study is the low number of subjects. Since black subjects tended to have a more robust increase in blood pressure in response to the saline infusion, additional subjects may have made the difference significant. Another limitation is the smaller percentage of women in the black group as compared with the white group.

In conclusion the hypertonic saline infusion caused an increase serum sodium, osmolality, and plasma volume. These acute changes caused an increase in

mean arterial blood pressure that was not different between the two groups. There were changes in both cardiac output and peripheral vascular resistance that seemed to contribute to the increase in mean arterial pressure.

Appendix A

LITERATURE REVIEW

Sodium and Blood Pressure

Mean arterial pressure is equal to the product of cardiac output and peripheral vascular resistance[18]. The positive relationship between these three factors indicates that a rise in blood pressure can be attributed to either a rise in cardiac output, peripheral vascular resistance, or both. While high sodium intake is associated with elevated blood pressure levels, it is not clear whether increased plasma osmolality or increased plasma volume are the cause; but it is most likely a combination of both factors.

Many epidemiological studies have established a link between chronically high dietary sodium intake and high blood pressure. Recently the Dietary Approaches to Stop Hypertension (the DASH diet) looked at the effect of sodium intake on blood pressure[1]. Two different diets were used; one was a diet that was high in fruits, vegetables, and low-fat dairy, often referred to as the DASH diet; the other was a diet that more closely resembled a typical American diet. Three sodium intake levels were studied; a high level of 150 mmol/d, and intermediate level of 100 mmol/d and a low level of 50 mmol/d. While there was variability between subjects, blood pressure was

reduced in a stepwise fashion as sodium level decreased.

He et al investigated the effect of changes in plasma sodium on blood pressure during a high and low salt diet in black and white, normotensive and hypertensive subjects[2]. The dietary interventions were either short or long term. The reduction in sodium intake reduced plasma sodium concentration significantly independent of the length of the intervention.. Long-term reduction in sodium intake caused decreased plasma sodium levels that were correlated with a reduction in systolic blood pressure indicating that the greater the reduction in plasma sodium, the greater the fall in systolic blood pressure.

Salt Sensitivity Defined

While the link between dietary sodium intake and blood pressure has been shown through many studies, there is not a uniform blood pressure response to changes in dietary sodium intake. Results from the DASH-sodium trail confirmed that there is a wide variability of individual blood pressure responses to sodium[5]. Salt sensitivity is a concept that helps to identify individuals that are responders to changes in dietary sodium intake. Individuals that are salt sensitive will have an increase in mean arterial blood pressure in response to an increase in sodium intake.

Weinberger et al devised a protocol to test salt sensitivity without the use a dietary intervention[7]. Subjects received a rapid increase in extracellular volume and sodium balance through a four-hour infusion of two liters of 0.9 percent saline. On

the following day sodium and volume were depleted by a 10 mmol per day sodium intake as well as the administration of fluorside, a diuretic. Blood pressures taken at the end of the saline infusion and the morning after the sodium depletion protocols were compared and a difference in mean arterial pressure of 10 mmHg or more was set as the criteria for salt sensitivity. They found that both hypertension and age were correlated with salt sensitivity.

One study attempted to link dietary and intravenous loading of sodium for the assessment of salt sensitivity. Sharma et al used the same protocol as Weinberger in order to intravenously administer and then deplete sodium in subjects[19]. It was compared to a dietary protocol in which subjects were randomly assigned a 7-day high salt, and a 7-day low salt diet. Salt sensitivity was defined as a decrease in mean arterial blood pressure ≥3 mmHg during the low salt diet or at the end of the sodium depletion period. There were significant correlations in diastolic and mean arterial blood pressure between intravenous and dietary protocols but the blood pressure responses to the intravenous protocol did not necessarily predict the response to the dietary protocol. The dietary protocol identified 8 subjects as salt sensitive, while the intravenous protocol identified only 5. However, all 8 subjects identified by the dietary protocol had a modest increase in blood pressure during the intravenous protocol. While this study did not find that the intravenous and dietary protocol could uniformly predict salt sensitivity, there were similarities between the blood pressure responses to both. Perhaps if 24-hour ambulatory blood pressures were taken during

the dietary protocol, instead of a 1-hour measurement at the end of each dietary protocol the results would have better validated the intravenous protocol.

Blood Pressure and Race

Black adults have a greater prevalence and earlier onset of essential hypertension as compared to white adults[8]. Recently, an epidemiological study done in the United States found that 38.8% of Non-Hispanic black adults had hypertension. This percentage is considerably higher than that of white and Mexican Americans, at 27.2 and 28.7% respectively. While the underlying mechanisms are not clear, this high prevalence may be due in part to the high prevalence of salt sensitivity in the African American population.

A further analysis of the DASH-sodium trials looked at the subgroups involved in the study separately[20]. As sodium intake decreased blood pressure decreased in all subgroups independent of age, ethnicity, hypertension status, and a number of other groups. However, African Americans had a significantly greater blood pressure response to changes in sodium intake than did non-African American subjects.

Morris et al investigated the effects of race and potassium on salt sensitivity[21]. After two weeks of a controlled, low sodium diet, all subjects, black and white, underwent a sodium load for four weeks. While on the low sodium diet, blood pressure did not differ between blacks and whites. During the high sodium diet phase,

both systolic and diastolic blood pressure increased significantly in black subjects as a group but not in white subjects. Salt sensitivity was defined as an increase of mean arterial blood pressure of 3mmHg or greater as a result of an increase in sodium. Salt sensitivity was found in 79% of black subjects but only in 36% of white subjects indicating a significantly higher prevalence of salt sensitivity in the black population.

Luft et al examined the cardiovascular and humoral responses to sodium administration in normotensive black and white men[4]. Subjects were observed during six different phases of sodium intake ranging from very low to very high. Both black and white subjects experienced an elevation in mean systolic and diastolic blood pressure, cardiac index, and stroke index with the high salt intake compared with the low salt intake. Black subjects demonstrated a greater increase in blood pressure at higher sodium intakes as compared with white subjects. This may help to explain the higher prevalence of hypertension in black Americans as compared with white Americans despite no differences in sodium intake.

Wright et al examined whether differences exist in sodium pump activity and blood pressure reactivity to sodium in normotensive and hypertensive black and white women[6]. All subjects were prescribed in random order, a 7-day high salt, and a 7-day low salt diet. Salt sensitivity was defined as an increase in mean arterial pressure from the low to high salt diet equal to or greater than 10 mmHg. Prevalence of salt sensitivity was similar between black and white subjects but the inability to detect differences may be due to the more rigid definition as compared with other studies.

Despite this finding normotensive and hypertensive black subjects had a greater increase in systolic blood pressure and higher sodium retention in response to the high sodium diet as compared with white subjects.

While many have speculated, the cause of the high prevalence of salt sensitivity in black adults is not clear-cut. It may be due to a higher vascular reactivity in black adults as compared with white adults. Light et al investigated the hemodynamic responses to laboratory stressors in black and white subjects[9]. They used a series of laboratory stressors including mental arithmetic, a reaction time task, and a forehead cold pressor test. The stressors elicited a similar increase in blood pressure for black and white subjects however; the black subjects demonstrated a higher total peripheral vascular resistance while the white subjects had greater increases in heart rate and cardiac output. This finding was consistent with both male and female subjects.

Stein et al used pharmacological α-adrenergic stimulator, phenylephrine, along with a cold pressor test, and a lower body negative pressure test to compare vasoconstriction through forearm blood flow responses in black and white subjects[10]. Isoproterenol, a β-agonist, was also used to measure vasodilation. The infusion of phenylephrine and the cold pressor test caused a significantly larger increase in forearm vasoconstriction in the black subjects while isoproterenol caused significantly smaller increases in vasodilation. During the cold pressor test, catecholamine spillover was similar between black and white subjects indicating that the higher vascular

resistance in the black subjects was not mediated by increased sympathetic nervous system activity.

Based on the hypothesis that a1-adrenergic receptor sensitivity may be elevated in black adults, Ray et al examined sympathetic vascular transduction during lower body negative pressure in young normotensive black and white subjects[11]. Muscle sympathetic nerve activity, blood pressure, heart rate, and forearm blood flow were measure during baseline and then during 4 minutes each of lower body negative pressure at -5, -15, and -40 mmHg in that order. During lower body negative pressure heart rate was significantly increased, blood pressure did not change, and forearm blood flow was significantly reduced in all subjects with no differences between black and white subjects. While there were no differences at rest, lower body negative pressure caused a greater increase in muscle sympathetic nerve activity in white subjects. This means that at a given level of sympathetic nerve activity, black subjects had greater forearm vascular resistance than white subjects, indicating an elevated sympathetic vascular transduction in black subjects.

Infusion studies

The effect of hypertonic saline on blood volume has been examined by a number of studies. Jarvela et al investigated the effect of hypertonic saline infusion on plasma and extracellular volume in healthy subjects[22]. A 7.5% hypertonic saline was infused into eight postmenopausal women over 30 minutes. Heart rate, cardiac index, serum osmolality, plasma sodium, plasma volume and extracellular and interstitial

water were measured throughout the infusion and for 60 minutes after. During the infusion, plasma volume and extracellular water significantly increased and remained elevated during the follow up period. Interstitial water decreased initially but then increased during the infusion. During the follow up period the interstitial water continued to increase and then declined during the last 20 minutes. Blood pressure, heart rate, and cardiac index all increased during the infusion. Serum osmolality and plasma sodium increased throughout the infusion and then declined during the follow up period. The hypertonic saline produced a hyperosmotic state, which caused expansion of plasma volume and extracellular water by drawing water from the intracellular space.

The hemodynamic effects of hypertonic saline in dogs were examined by Rowe et al[23]. Blood pressure, cardiac output, stroke volume, total peripheral resistance, heart rate, and hematocrit were measured at baseline and then during the infusion of a 30% hypertonic saline. While there was no significant increase in blood pressure heat rate, stroke volume, and cardiac output increased, while total peripheral resistance decreased. There was also a decrease in hematocrit indicating an increase in plasma volume. The effects seen may be attributed to hemodilution, which was indicated by the decrease in hematocrit, and caused by the hyperosmotic state that the hypertonic saline infusion produced.

Appendix B

UNIVERSITY OF DELAWARE

HUMAN PERFORMANCE LABORATORY

MEDICAL HISTORY QUESTIONNAIRE

I. Personal Information:

Name/Last First Middle Initial

Age Sex E-mail address Phone Number

II. Medical History:

A. ILLNESSES

Please check if you have had any of the following:		Dates
1. Heart attack	_____	_____
2. Asthma	_____	_____
3. Epilepsy	_____	_____
4. Back problems	_____	_____
5. Lung disease	_____	_____
6. Stroke	_____	_____
7. Diabetes	_____	_____

8. Heart murmur _____ _____

9. Allergies _____ _____

10. Cancer _____ _____

B. Symptoms

During the last 12 months have you experienced:

	YES	NO
1. High blood pressure	_____	_____
2. Swelling of hands/feet	_____	_____
3. Pain or cramps in legs	_____	_____
4. ECG abnormalities	_____	_____
5. Chest pain/pressure	_____	_____
6. Shortness of breath	_____	_____
7. Numbness/tingling in arms, hands, or legs	_____	_____
8. Significant weight fluctuation (> 5 lb.)	_____	_____

C. QUESTIONS

1. Please list any serious or chronic illness that you are aware of: _____

_____

_____

_____

2. Please list any allergies to medications, foods, or other substances: _____

_____

_____

_____

3. Do you currently use tobacco products? YES NO

4. Please list any medication you have been on or presently take:

Type of Medication	Dosage	How Long	Reason

D. HOSPITALIZATIONS

List the dates, and reasons for hospitalizations for any significant illness/injury.

	Date	Diagnosis
1.		
2.		
3.		
4.		
5.		

III. Musculoskeletal History:

Do you have any injuries or orthopedic limitations that may affect your **FULL** participation in this study? **YES NO**

If yes, please explain: _____

_____

Appendix C.

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE PAR-Q

YES NO

1. O O Has your doctor ever said you have heart problems or a heart murmur?

2. O O Do you frequently have pains in your heart and chest?

3. O O Do you often feel faint or have spells of severe dizziness, passed out, or have persistent rapid or irregular heart beat?

4. O O Has a doctor ever said your blood pressure was too high (Systolic pressure greater than or equal to 160mmHg, or diastolic pressure greater than or equal to 90rmnHg)?

5. O O Do you smoke cigarettes?

6. O O Do you have Diabetes?

7. O O Do you have a family history of heart disease in parents or siblings prior to the age of 55?

8. O O Have you ever been told you have high cholesterol?

9. O O Is there any physical reason not mentioned here why you should not perform physical exertion?

Appendix D

INFORMED CONSENT FORM

Research Study: Sympathetic – Osmotic Interactions in Humans
Investigators: William B. Farquhar, PhD and Michael E. Stillabower, MD

SUBJECT NAME: _____

1. PURPOSE / DESCRIPTION OF THE RESEARCH

You are being asked to participate in a joint research study conducted by the University of Delaware and Christiana Care Health Services' Cardiovascular Research Program. The purpose of this study is to examine some of the factors that control blood pressure in normal healthy adults. There is evidence linking sodium chloride (i.e., salt) to high blood pressure. However, the mechanisms underlying this link are not completely understood. This study is being performed to determine if sodium chloride in the blood (i.e., circulating) stimulates the nervous system (i.e., sympathetic nervous system activity), and if this is what causes the increase in blood pressure.

You will be one of 36 healthy subjects (men and women) between the ages of 21-44 years old that will be recruited for the study. A special effort will be made to ensure inclusion of black adults in this study. This will allow us to determine if the blood pressure response to sodium in the blood differs in black adults compared to other racial groups. The full study will require 3 visits spread over a period of 2-3 months. You will be asked to not eat for 12 hours prior to the study, and abstain from alcohol, caffeine, and exercise for at least 12 hours.

<u>First Session:</u>
The first session will be about 2 hours in length. In order to determine if you qualify to be in the study, the following information will be obtained: a complete medical history using a questionnaire, a PAR-Q questionnaire (physical activity readiness questionnaire), height, weight, a resting electrocardiogram, resting blood pressure, a body fat assessment using skinfold calipers, and a blood sample (obtained with a

needle from a vein in the arm). The blood sample will be used to obtain an assessment of liver function, a lipid profile, a complete blood count, glucose, sodium and other chemicals in the blood, and kidney function tests. About 1 teaspoon will be sampled. In addition, a moderate fitness test will be performed on a stationary bicycle. Prior to this test, 10 electrodes will be placed on your chest. During this test, your heart rate and blood pressure will be monitored while you pedal at about 60 revolutions per minute. Every 3 minutes the resistance will be increased. The test will be stopped before you reach maximal effort. The test will also be stopped earlier if you have any abnormal symptoms such as chest pain, have an abnormal blood pressure response, or have an abnormality on the electrocardiogram. It is normal to breath heavy and sweat during a fitness test. All information will be reviewed by the investigators (including a physician), and only those with no signs or symptoms of disease, normal blood work, a normal resting electrocardiogram, and normal blood pressure will be accepted into the study. Exclusion criteria based on the PAR-Q include any affirmative answer to questions 1-4, and/or 9, and not more than one affirmative answer to questions 5-8. In addition, you will be excluded if you are obese (body mass index greater than 30), use tobacco, are currently taking prescription medications, or – for the women – pregnant (an over-the-counter pregnancy test will be performed). The investigators will discuss the results of these tests with you, and upon request, will forward the results to your primary care physician. In the event that one of the test results is abnormal, you will be referred to your primary care physician for follow-up.

Second Session:

This session will be about 4.5 hours in length. You will have 5 electrodes placed on your chest to monitor heart rate, attachment of a cuff around the upper arm and finger to measure blood pressure, elastic-like bands will be placed around the chest and stomach to assess breathing rate, and a pressure cuff will be placed around the thigh and ankle along with an elastic band around the calf to assess leg blood flow. In addition, an intravenous catheter (i.e., a small needle encased in a flexible tube that is inserted into a vein) will be placed into each forearm. One of these catheters will be used to put saline (i.e., sterile salt water) in the vein, and the other will be used to obtain blood samples. In order to assess nervous system activity ("sympathetic nerve traffic"), a fine wire needle will be inserted through the skin to record from the peroneal nerve (located under a bony prominence on the side of the lower leg, just below the knee). The skin will be wiped with alcohol prior to insertion. In some, but not all subjects, we will estimate the output of the heart instead of nervous system activity. This technique is referred to as "CO_2 rebreathing". This is done by having you breathe with a mouthpiece and valve in place. The first step is to breathe in normal room air, while we measure the carbon dioxide and oxygen content of your exhaled air (this takes about 5 minutes). The second step is to breathe in a gas mixture containing a higher concentration of carbon dioxide and oxygen (for about 15 seconds), while we assess the carbon dioxide and oxygen content of your exhaled air.

This will be repeated 3-5 times. We will then put saline into the vein over the next hour. The type of saline put into the vein will be either 0.9% saline or 3% saline (containing a higher concentration of salt). This order will be determined by flipping a coin; therefore if 0.9% saline is put in the vein during the second visit, 3% saline will be put in the vein during the third visit. Blood samples will be obtained while the saline is being put into the vein. The total volume of blood to be sampled will be 60 mL (about 4 tablespoons). The total volume of fluid to be put in the vein will depend on your body weight, but for most will be about 400-500 mL (for reference, a 12 ounce soft drink contains 355 mL). Nervous system activity will be assessed while the saline is being put into the vein (or alternately, the output of the heart using the CO_2 rebreathing technique will be assessed while the saline is being put into the vein).

The heart rate and blood pressure to deep breathing (i.e., paced breathing) will be assessed while the saline is being put into the vein. Breathing rate will be voluntarily controlled by listening to a tape (example: in 2, 3, out 2, 3, in 2,3, out 2,3, etc). The heart rate and blood pressure responses to a brief change in blood pressure will be induced by a forceful expiration (this is known as a Valsalva maneuver). Resistance will be provided by breathing through a tube for 15 seconds. This maneuver will also be performed while the saline is being put into the vein. Following the completion of the protocol, your heart rate, blood pressure and respiration rate will be monitored for 1 additional hour to ensure that you do not experience any adverse effects.

Third Session:

The third visit will be identical to the second, except that a different concentration of saline will be used (that is, if 0.9% saline was used during the second visit, 3% saline will be used during the third visit).

2. CONDITIONS OF SUBJECT PARTICIPATION

Information obtained from this study will be kept strictly confidential. You will not be individually identified, except by subject number known only to the investigators. The funding agency – the National Institutes of Health – may request access to the data during an audit, but they are bound by the same level of confidentially as the investigator. All data stored as paper files or on computer disk will be kept in a locked cabinet for three years, and then will be destroyed. While the results of this research may be published, your name or identity will not be revealed.

In the event of physical injury as a direct result of these research procedures, you will receive emergency medical treatment. If you require additional medical treatment, you will be responsible for the cost. You are free to withdraw from the study at any time without penalty.

3. RISKS AND BENEFITS

First Session:

There are no known risks associated with obtaining your height, weight, resting electrocardiogram, and resting blood pressure. You may have pain and/or bruising at the site where blood is taken during the first session, and there is a small risk of infection. Fainting sometimes occurs during or shortly after blood is drawn. There may be minor discomfort associated with the placing and removing of electrodes. The risks associated with exercise / fitness testing are low. However, there exists the possibility of certain changes occurring during the exercise test. These include abnormal blood pressure, fainting, abnormal heart beats, and in rare instances, heart attack, stroke, and death. In order to minimize these risks, a 12-lead electrocardiogram and blood pressure will be monitored throughout, and the test will be immediately stopped if any abnormalities develop. You can stop the test at any time if have feelings of fatigue or any other discomfort. Shortness of breath is a normal response to exercise testing, and will be experienced during the test. Standard procedures and guidelines issued by organizations such as the American College of Sports Medicine and the American Heart Association will be followed.

Second and Third Sessions:

There are no known risks associated with measuring heart rate, blood pressure, or respiration. There can be very minor discomfort when the blood pressure cuff is inflated. There is no known risk to measuring blood flow in the leg; once again, there can be very minor discomfort when the pressure cuffs around the thigh and ankle are inflated. You may have pain and/or bruising at the site where the intravenous catheters are placed.

In order to find the *general location* of the peroneal nerve, mild external electrical stimuli will be applied using a wand-like device; this may cause minor discomfort. In order to find the *precise location* of the peroneal nerve, mild electrical stimuli will also be used after the fine wire needle is inserted through the skin; this may also cause minor discomfort, such as muscle twitches and a pins and needles sensation. This electrical stimulation is only used to locate the peroneal nerve, once the nerve is found

the stimuli is turned off and the fine wire needle (connected to a measuring device) is used to record nervous system activity. There may also be mild discomfort when the small needle is inserted through the skin, however, this needle is very small, and many subjects do not feel the insertion. It is possible that feelings of muscle weakness and/or pins and needles sensations can be felt after the procedure. There is no specific treatment for these sensations, and in the small number of volunteers that have experienced them, they have disappeared spontaneously within a few days. It is generally thought that the risks of side effects are minimized when no more than 1 hour is used to locate the peroneal nerve with the small needle; therefore, in this study, this time limit will be strictly enforced. There is also a small risk of infection at the site where the fine wire needle is inserted. There are minimal risks associated with breathing through a valve and with breathing a higher concentration of carbon dioxide and oxygen. While unlikely, it is possible that you may feel dizzy or lightheaded for a very brief period of time during or after this procedure.

There are some risks associated with putting 0.9% saline and 3% saline into the vein. When the 3% saline is put in the vein, it is anticipated that the concentration of sodium in the blood will rise. Potential risks (while low in healthy subjects) associated with this procedure include: damage to the vein, blood dilution, spasm or contraction of the vein, high blood pressure, excess fluid in the lungs or in the brain, volume overload (to much fluid in the body), and heart failure. Symptoms of these conditions include discomfort in the arm where the flexible catheter is placed, shortness of breath, a headache, and / or fluid accumulation in the arms, legs, or lungs. However, occasional blood sampling – with the immediate testing of the sample for sodium concentration – will allow the investigators to stay within a normal "physiological" range. In the event that the sodium concentration rises to a higher than normal level, the procedure will simply be stopped. Sodium concentration will return on its own to normal values. In the event that you experience any adverse symptom during the procedure, such as shortness of breath, the procedure will be stopped. Following termination of the protocol, you will continue to be monitored for 1 hour to ensure that any or all symptoms disappear. If further medical follow-up is needed, you will be transported to the hospital.

There are minimal risks associated with the performance of a "valsalva maneuver" (forced expiration against a resistance). You may feel dizzy or lightheaded for a very brief period of time after this procedure.

There may be no benefit to you for participating in this research study. The data collected during the screening session includes fitness level and blood work. This information will be provided to you upon completion of the study. In addition, the study may provide new, useful information regarding the control of blood pressure in normal healthy adults.

4. FINANCIAL CONSIDERATION

All subjects will receive $50.00 to offset the cost of transportation, etc. for full completion of the study. Those who choose to complete half the study will receive a $25.00.

5. CONTACTS

Any questions regarding the study can be directed to William B. Farquhar, PhD, Assistant Professor, Department of Health and Exercise Sciences (302-831-6178) or Michael E. Stillabower, MD, Director of Cardiovascular Research, Christiana Care Health Services, Inc. (302-733-2658). Questions regarding the rights of individuals who agree to participate in this research study may be directed to Dr. T.W. Fraser Russell, Vice Provost for Research, University of Delaware (302-831-4007).

In the event of an emergency, you can contact the following 24-hour telephone number: Michael E. Stillabower, MD, Phone: (302) 733-2658.

6. SUBJECT'S ASSURANCES

I have read the above informed consent document. The nature, demands, risks and benefits of the project have been explained to me. I knowingly assume the risks involved, and understand that I may withdraw my consent and decide to not be in this study at any time without penalty or loss of benefit to myself. A copy of this consent has been given to me.

7. CONSENT SIGNATURES

Subject's Signature: _____ Date: _____

Subject's Name (printed): _____ Date: _____

I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with being in this research study, have

answered any questions that have been raised, and have witnessed the above signature. I have provided the subject with a copy of this informed consent document.

Signature of the Investigator: _____ Date: _____

Appendix E

SYMPATHETIC – OSMOTIC INTERACTIONS IN HUMANS

SCREENING CHECKLIST

Subject_____

Date _____

BEFORE SUBJECTS ARRIVES

____ Cart is warmed up and calibrated

____ Valves for CO2 rebreathing and exercise test are set up

____ Electrodes and alcohol swipes for ECG

____ Make sure there is paper in ECG machine

____ Tubes for blood sample are set up

____ New folder with med history, PAR-Q, blood sheet, exercise test form, informed consent,

subject instructions, protocol checklists, screening checklist, and subject payment form.

PAPER WORK

____ Subject initialed and signed informed consent

____ Subject filled out medical history questionnaire and PAR-Q

____ Obtain height and weight

Height:_____

Weight: _____

____ Hip to waist ratio

Hip_____ Waist_____ Ratio_____

BODY FAT ASSESSMENT

Site	Trial 1	Trial 2	Trial 3	Mean
1	_____	_____	_____	_____
2	_____	_____	_____	_____
3	_____	_____	_____	_____
			SUM	_____

Body Fat _____%

RESTING VALUES

____ Resting ECG

____ Resting BP (two trials)

BP: ___/____mmHg

BP: ___/____mmHg

Submaximal bike test (fill out next form)

____ Use submaximal bike protocol

____ Age predicted max * 85% for exercise end point

CARDIAC OUTPUT PRACTICE

____ Teach maneuver

____ Bag Volume used_____L

____ PCO2 used _____%

END OF SCREENING

____ Subjects has instruction for protocol

____ Subject has a signed copy of informed consent

____ (females only) Estimation of early follicular phase for testing

_____ Dates

____ Subject is scheduled for infusion

_____ Date

BLOOD SAMPLING

____Tiger top tube, Lavender top tube, 5mL red top tube, Green tube

____ Spin tiger top, red top, and green top – DON'T SPIN LAVENDER TOP!

____ Run Na/K/Cl and osmolality in lab

____ Bring lavender top/tiger top to hospital

Appendix F

SYMPATHETIC – OSMOTIC INTERACTIONS IN HUMANS

CARDIAC OUTPUT PROTOCOL CHECKLIST

Subject ID number: _____

Date: _____ Temp_____

Session: 2nd / 3rd (circle one) Humidity_____
Infusion: 0.9% saline / 3% saline (circle one)

Subject: Weight _____ Total saline infused _____

Informed Consent on File: Yes or No (circle one)

Bag volume/ PCO2 used during practice
_____ Liters
_____% CO2

Before subject arrives

Set Up

____ 3 pre-chilled, specially prepared EDTA-containing tubes (for NE)
(separate plasma within 30 minutes using a refrigerated centrifuge)
(freeze plasma)

____ 3 EDTA 10mL tubes, lavender top for Renin, Ang II, and Aldos (centrifuge immediately
freeze plasma)

____ 5 No additive 5mL tubes, red top Na/K/Cl

____ 5 additional EDTA-containing 5 mL, tubes, lavender top for Hct, Hb and AVP
(transfer to micro-capillary tube and spin using small centrifuge, place tube on hematocrit reader)

____ 5 Sodium heparin tube, 5 mL green top tubes (for Osmolality)
spin immediately, run on Advanced Instruments Analyzer

____ Tubes labeled

____ Windaq set up

___ Warm-up metabolic cart for 30 minutes

____ Calibrate cart: volume and gas calibration

BEFORE INFUSION

Initial Urine Collection

____ Take upon arrival
____ Start stop watch
____ Specific Gravity: _____
____ Subject sufficiently hydrated? Y / N
____ Pregnancy test for women: Positive / Negative (circle one)

Breath sounds and Heart Auscultation

____ Taken before putting on equipment
____ Normal/Abnormal (circle one)

Void Bladder

____ Empty bladder before being fit with all equipment
____ Re-start stop watch
____ Open windaq, confirm sample rate of 500 Hz

File Name:	_____
channel 1	Respiration
channel 2	ECG
channel 3	beat-by-beat blood pressure
channel 4	strain gauge (confirm sensitivity at 1%)

Equipment / Instrumentation

____ ECG (5 electrodes)

____ Respiratory Bands

____ Place two I.V. lines

____ 2 leg cuffs + strain gauge (strain gauge size:_____)

____ Calibrate strain gauge

____ Finger cuff with calibration arm cuff – make sure signal is good

____ Automatic blood pressure cuff

____ Pulse oximeter

Baseline Data Collection

***** Turn down lights*****

____ Blood pressure with Dinamap x 2 _____/_____, _____/_____

____ Cardiac Output

____ 5 minute VCO2

____ end-tidal CO2 / rebreathing maneuver

____ Blood Draw from I.V. line (confirm that subject has been supine for at least 30 min)

____ Draw 40 mL

____ (2) 10 mL EDTA-Sodium Metabisulfite (NE)

____ (1) 5 mL lavender top (AVP, Hct, Hb)

____ (1) 5 mL EDTA red top tubes (Na/K/Cl) **ONLY NEED 2-3 ML!**

____ (1) 5 mL green top (osmolality)

____ (1) 10 mL lavender top tube (Aldos, Renin, AngII)

____ Start Finometer – RTF calibration

____ 5 minutes of paced breathing (0.25Hz, RSA assessment)

____ 2 minutes of limb blood flow during paced breathing (inflate ankle cuff 1 minute prior)

Start of Infusion

____ Start stop watch

____ Note beginning of infusion on Windaq

14 minute

____ Draw 15 mL

____ (1) 5 mL lavender top (Hct and Hb)

____ (1) 5 mL green top (osmolality)

____ (1) 5 mL red top (Na/K/Cl) **ONLY NEED 2-3 ML!**

18 minute

____ 5 minutes of paced breathing

____ Limb Blood Flow for 2 minutes during paced breathing (inflate ankle cuff 1 minute prior)

23 minute

____ Blood pressure with Dinamap x 2 _____/_____, _____/_____

____ Heart Rate _____bpm

____ Cardiac Output

____ End-tidal CO2 / rebreathing maneuver

29 minute

____ Draw 40 mL

____ (2) 10 mL EDTA-Sodium Metabisulfite (NE)

____ (1) 5 mL lavender top (AVP, Hct, Hb)

____ (1) 5 mL EDTA red top tubes (Na/K/Cl) **ONLY NEED 2-3 ML!**

____ (1) 5 mL green top (osmolality)

____ (1) 10 mL lavender top tube (Aldos, Renin, AngII)

33 minute

____ 5 minutes of paced breathing

____ Limb Blood Flow for 2 minutes during paced breathing (inflate ankle cuff 1 minute prior)

38 minute

____ Blood pressure with Dinamap x 2 _____/_____, _____/_____

____ Heart Rate _____ bpm

____ Cardiac Output

____ end-tidal CO2 / rebreathing maneuver

44 minute

____ Draw 15 mL

____ (1) 5 mL lavender top (Hct and Hb)

____ (1) 5 mL green top (osmolality)

____ (1) 5 mL red top (Na/K/Cl) **ONLY NEED 2-3 ML!**

50 minute

____ Limb Blood Flow for 2 minutes (inflate ankle cuff 1 minute prior)

____ 5 minutes of paced breathing

55 minute

____ Draw 40 mL

____ (2) 10 mL EDTA-Sodium Metabisulfite (NE)

____ (1) 5 mL lavender top (AVP, Hct, Hb)

____ (1) 5 mL EDTA red top tubes (Na/K/Cl) **ONLY NEED 2-3 ML!**

____ (1) 5 mL green top (osmolality)

____ (1) 10 mL lavender top tube (Aldos, Renin, AngII)

____ Blood pressure with Dinamap x 2 _____/_____, _____/_____

____ Heart Rate _____ bpm

____ Cardiac Output

____ VCO2 for 2 minutes

____ end-tidal CO2 / rebreathing maneuver

____ MARK TOTAL INFUSION TIME _____

____ Total amount of saline infused _____

POST INFUSION

1. Monitor Subject

____ Breath Sounds and Heart Auscultation
Normal / Abnormal (circle one)

2. Urine Collection

____ End stop watch – Time _____
____ Collect and measure:
Total volume _____
Specific Grav. _____

Post Infusion BP: _____ _____

Post Infusion HR:_____ _____

____ Confirm subject is asymptomatic

____ Have subject fill out payment form

3. Blood Samples

____ Collect capillary tubes for Hct from lavender top tubes
____ Centrifuge tubes at 10 degrees C
____ Pipette plasma or serum into labeled test tubes

Green Top at 1, 2, 3, 4, and 5: Osmolality
Lavender Top at 1, 3 and 5: Hct and AVP
Lavender Top at 2 and 4: Hct
7mL Red Top at 1, 3, and 5: Na/K/Cl and Aldosterone
5mL Red Top at 2 and 4: Na/K/Cl
5mL Red Top at 1, 3, and 5: Renin
10mL Red Top at 1, 3, and 5: NE

Freezer Tubes:

Blood at 1, 3, and 5 From -
Lavender top for AVP
5 mL red top for Renin
7 mL red top for Aldosterone
10 mL red top for NE (2 tubes for each time)

4. File Transfer

____ File transferred from Finapres to computer
File location _____
____ Backup windaq file to CD and back up hard drive

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