THE RELATIONSHIP BETWEEN HABITUAL SODIUM AND POTASSIUM INTAKE ON VASCULAR FUNCTION IN HEALTHY, OLDER ADULTS

by

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ABSTRACT

Hypertension (HTN) is a significant Public Health problem and the risk of an elevated blood pressure increases greatly with advancing age. Vascular dysfunction may precede HTN, characterized by an increase in inflammation, thrombosis, coagulation, proliferation, and constriction of the vasculature. Dietary factors can influence the development of HTN and the effects of a high sodium/low potassium diet on increasing blood pressure are well known. While these two minerals have been studied extensively in relation to HTN, it is becoming clearer that they may impact the vasculature prior to a change in blood pressure. Recently, the sodium:potassium excretion ratio has been used as a marker of cardiovascular health. The purpose of this study was to cross-sectionally examine the relationship between sodium and potassium intake and vascular function in a healthy, aged population free of hypertension. Thirty subjects (16 M, 14 F) with an average age of 62 $\pm$ 1 years were recruited for this study. Subjects recorded their dietary intake for three days and collected their urine for 24 hours on the final day. Following the last day of self-collection, subjects came to the lab for a vascular assessment, including measurements of pulse wave analysis (PWA), pulse wave velocity (PWV), and brachial artery flow-mediated dilation (FMD). Urinary levels of sodium and potassium were determined. Dietary analysis revealed that subjects consumed a higher level of

sodium (3,187 ± 169 mg) and a lower level of potassium (3,120 ± 177 mg) than recommended. The primary variable, the sodium:potassium excretion ratio did not correlate with any of the vascular measurements, nor did sodium or potassium intake, or sodium or potassium excretion. However, a higher sodium excretion was associated with higher systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP). When comparing men to women, there was a significant difference in augmentation index (AIx) that was to be expected given height differences. In conclusion, in this sample of healthy, aged adults, the sodium:potassium excretion ratio and thereby sodium and potassium intake, does not appear to have an effect on vascular function. However, as supported by previous literature, sodium excretion related to blood pressure. Therefore, because of the small sample size, more extensive research needs to be performed to examine the relationship between the sodium:potassium excretion ratio and vascular health measurements in a larger population with a greater range of sodium and potassium intakes.

Chapter 1

INTRODUCTION AND BACKGROUND

1.1 Statistics and Definition

Cardiovascular disease stands as the leading cause of death in the United States (CDC, 2009), and it encompasses high blood pressure (HBP) and coronary heart disease (CHD) (Roger et al., 2012). An estimated 82.6 million Americans have one or more types of CVD and of these, 40.4 million are ≥ 60 years of age (Roger et al., 2012). CVD is often based in HTN, which is characterized by chronic high pressure in the vasculature. In the United States, 77.9 million people (33%) over the age of 20 years have HTN (Go et al., 2013). Projections using recent data show that by the year 2030, there will be a 9.9% increase in prevalence, or an additional 27 million people with HTN (Roger et al., 2012). HTN often precedes many forms of cardiovascular disease as approximately 69% of people who have a first heart attack, 77% who have a first stroke, and 74% who have congestive heart failure all had a blood pressure higher than 140/90 mm Hg (Roger et al., 2012). Therefore, HTN is a major public health concern due to its high prevalence.

By definition, HTN is a repeated high pressure exerted against the arterial walls by blood, equating to a systolic blood pressure (SBP) of ≥ 140 mmHg and/or a diastolic blood pressure (DBP) of ≥ 90 mmHg. An individual is also

considered hypertensive if they are currently taking anti-hypertensive medications or if they have been told that their blood pressure is high on two separate occasions (Joint National Committee, 2004). There are two types of HTN: primary and secondary. Essential HTN, otherwise known as primary HTN, or HTN of an unknown etiology, accounts for over 95% of cases of HTN and represents a culmination of influences from genetically based diseases to environmental factors (DiPiro et al., 2002; Carretero and Oparil, 2000). Secondary HTN occurs in less than 5% of the population and is defined as having identifiable causes such as renal dysfunction (DiPiro et al., 2002). Blood pressure is classified into four groups:

Classification	**Systolic Blood Pressure**	**Diastolic Blood Pressure**
Normotensive	< 120 mmHg	< 80 mmHg
Prehypertensive	120 – 139 mmHg	80 – 89 mmHg
Stage 1 Hypertension	140 – 159 mmHg	90 – 99 mmHg
Stage 2 Hypertension	≥ 160 mmHg	≥ 100 mmHg

(Joint National Committee, 2004)

Discerning the type and degree of HTN is extremely important when determining methods for improvement.

1.2 Epidemiology and Risk Factors in Hypertension

1.2.1. Epidemiology

When considering differences amongst populations with HTN, gender and age must be evaluated. Research has shown an increased prevalence of HTN in women after the age of 65 that is largely due to menopause (Roger et al., 2012). Overall, there is a 71.7% increase in the percentage of women with HTN from the 20-34 age group to the 75 and older age group, while there is only a 55.6% increase in the percentage of the population with HTN for males (NHANES, 2008). Age has a more profound impact on incidence of HTN than sex (Kearney et al., 2005). The NHANES 2007-2010 found that 67.4% of adults over 65 have HTN (Go et al., 2013). In addition, the prevalence of HTN is 8 times greater in older adults aged 75 or older compared to their young counterparts aged 20-34 (NHANES, 2008). Congruently, population projections for the year 2030 and 2050 show an increase in the total population of older adults. In the year 2030 there is a projected 7.2% increase in older adults over the age of 65 and a projected 2.4% increase in older adults over the age of 85 (US Census Bureau, 2010). The likely increase in the number of older adults in combination with the known increase in HTN with aging reveals the importance of discovering and implementing treatment methods and preventative medicine for older adults so that aged populations may maintain an optimal overall health.

1.2.2. Risk Factors

In addition to the listed epidemiological differences, there are also known risk factors associated with HTN. There is an associated risk of HTN in African Americans (40% more likely than their Caucasian counterparts), sedentary lifestyles, smoking, being overweight or obese, alcohol consumption, stress, and family history (CDC, 2010; Beunza et al., 2007; Jean-Michael et al., 2002; Hall, 2003; Huntgeburth et al., 2005; Zimmerman and Frohlich, 1990; Stamler et al., 1979). Likewise, nutrition plays a large role in the maintenance of blood pressure. Specifically, sodium and potassium intake affect blood pressure by increasing or decreasing pressure, respectively (Sacks et al. 2001; INTERSALT, 1988; Cook et al., 2007 and 2009; Sica et al., 2002; Whelton et al., 1997; Trials of Hypertension, 1997).

1.3 Regulation of Blood Pressure by the Renin-Angiotensin Aldosterone System

Blood pressure is largely regulated by the kidneys and the renin-angiotensin aldosterone system (RAAS). This system allows the kidneys to respond effectively to events such as a hypotensive environment or sodium restriction by increasing blood pressure to maintain homeostasis.

The kidneys initially release the enzyme renin which converts angiotensinogen to angiotensin I, which is then converted to angiotensin II, mediated by the angiotensin-converting enzyme (ACE). Angiotensin II stimulates

the secretion of aldosterone from the adrenal glands. Angiotensin II and aldosterone together contribute to sodium reabsorption and potassium excretion in the kidneys, leading to water retention and increases in blood pressure. When excess sodium is consumed, angiotensin II levels are low and subsequent aldosterone secretion is also low. Conversely, when too little sodium is consumed, angiotensin II levels are high and subsequent aldosterone secretion is also high (Widmaier et al., 2006).

It is proposed that the RAAS can alter vascular function by angiotensin II. Angiotensin II is a potent vasoconstrictor and regulator of blood pressure and water and electrolyte balance (Guyton, 1981). Infusion of angiotensin II increases pulse wave velocity (PWV), augmentation index (Aix), and aortic pulse pressure (PP) (Rehman et al., 2001; Wilkinson et al., 2001). Likewise, aldosterone can affect the vasculature, but only in presence of high sodium (Cailar et al., 2010).

In regards to diet, the intake of sodium can influence RAAS activity. In the presence of prolonged sodium intake of less than 115 mg/d, the RAAS is maximally activated (Laragh et al., 1972), whereas about 690 mg/d of sodium causes inhibition of RAAS with half-maximal stimulation of renin (Laragh et al., 1972). At 11,500 mg/d, release of aldosterone is almost completely suppressed (Laragh et al., 1972).

In aging, the effects of sodium intake on vascular health are more sensitive and responsive. With aging, phenotypic changes of the aortic smooth

muscle cells occur due to an increased amount of collagen and subsequent arterial stiffness (Safar, 2005). This change is partly due to an age-related increase in sodium sensitivity and endothelial dysfunction (Safar, 2005). Under the influence of angiotensin II and aldosterone, a high salt diet causes an increase in the number of attachments between smooth muscle cells and an increased development of collagen. This change causes an increase in vascular stiffness and an increase in early reflected waves, independent of mean blood pressure (Safar, 2005). This is supported by work in a rat model that showed there were more structural alterations of the arteries with a high sodium diet compared to a low sodium diet (Tobian, 1991). Likewise, in HTN, it has been found that increased sodium intake causes inadequate suppression of aldosterone, consequently causing impaired ventricular systolic and diastolic function (Schlaich et al., 1999). With aging, it appears that structural alterations occur in the vasculature, contributing to vascular dysfunction.

1.4 The Endothelium

The endothelium is important in the regulation of blood pressure. The endothelium is the largest organ in the human body. It is a one-cell thick wall providing the interface between the inner lumen and the smooth muscle layer of the artery. Albeit on such a small scale, the endothelium has profoundly important duties. Through the release and regulation of various factors, the endothelium is responsible for maintenance of vascular homeostasis by

controlling vascular tone, balancing blood fluidity, thrombosis and coagulation, and controlling of the vascular inflammatory process (Widlansky et al., 2003). Factors involved in maintenance of vascular tone include nitric oxide (NO), prostaglandins, endothelial hyperpolarizing factor, endothelin-1, angiotensin II, and c-type natriuretic peptide (Widlansky et al., 2003). Factors involved in balancing blood fluidity and thrombosis include NO, tissue plasminogen activator, thrombomodulin, prostaglandins, plasminogen activator inhibitor-1, and von Willibrand's factor (Widlansky et al., 2003). Factors involved in control of the vascular inflammatory process include monocyte chemotactic factor-1, adhesion molecule expression, interleukins 1, 6, and 18, and tumor necrosis factor (Widlansky et al., 2003).

NO, the major vasodilatory factor, is involved in maintenance of vascular homeostasis, and specifically prevention of the development of atherosclerosis. NO is synthesized by endothelial nitric oxide synthase (eNOS), using L-arginine as a substrate (Bredt and Snyder, 1994). In the presence of NADPH, BH_4 and oxygen, eNOS catalyzes the conversion of L-arginine to NO (Bredt and Snyder, 1994). NO causes vasodilation and assists in inhibition of platelet aggregation, leukocyte adhesion, smooth muscle cell proliferation, and inflammation (Bredt and Snyder, 1994). Each of these influences is anti-atherosclerotic, as they help protect the endothelium. NO is also important in maintaining the compliance of our vascular system, which is important in reducing the work of the heart and

thereby matching the ventricular work and the vasculature (Wilkinson et al., 2002).

In the vasculature, in a favorable, homeostatic environment, vasodilation is promoted, there are antioxidant effects, anti-inflammatory effects, anticoagulant effects, and profibrolytic effects (Widlansky et al., 2003). There is also inhibition of leukocyte adhesion and migration, inhibition of smooth muscle cell proliferation and migration, and inhibition of platelet aggregation and adhesion (Widlansky et al., 2003). If this homeostatic environment is altered, malfunctioning of the endothelium, or "endothelial dysfunction" occurs. Endothelial dysfunction is characterized by an impaired bioactivity of endothelial derived NO due to decreased synthesis of NO and/or a decreased bioavailability of NO (Feletou and Vanhoutte, 2006). In regards to decreased bioavailability of NO in the endothelium, oxidases produce superoxide, a free radical that reacts very quickly with NO (Carlos et al., 1998; Hamilton et al., 2001; Feletou and Vanhoutte, 2006). NO will more readily react with superoxide, instead of diffusing into the smooth muscle layer (Feletou and Vanhoutte, 2006). When it reacts with superoxide, the radical peroxynitrite is produced and continued damage can occur (Feletou and Vanhoutte, 2006). In addition to decreased bioavailability of NO, there may also be decreased synthesis of NO, due to uncoupling of eNOS. Tetrahydrobiopterin (BH_4) is an important cofactor for eNOS in the production of NO. If BH_4 is oxidized by superoxide or peroxynitrite, BH_4 becomes unavailable, and eNOS is uncoupled (Landmesser et al., 2003).

eNOS then becomes a superoxide generator because it cannot produce NO (Landmesser et al., 2003). This results in a decrease in NO production. Asymmetric dimethylarginine (ADMA) is an endogenously produced methylated arginine that can also inhibit eNOS and lead to uncoupling (Landmesser et al., 2003). Increased superoxide levels upregulate type I protein arginine *N*-methylarginine (PMRT), the enzyme that synthesizes ADMA, while simultaneously downregulating dimethylarginine dimethylaminohydrolase (DDAH), the enzyme that degrades ADMA, resulting in increased levels of ADMA (Landmesser et al., 2003; Sydow and Munzel, 2003). ADMA then inhibits eNOS resulting in a reduction in NO (Landmesser et al., 2003). Therefore, increased levels of oxidative stress can lead to a disruption of vascular homeostasis leading to endothelial dysfunction.

Endothelial dysfunction can contribute to development of atherosclerosis by adoption of a pro-thrombotic phenotype in the endothelial cells, and/or an increased expression of leukocyte chemotactic factors, adhesion molecules, and inflammatory cytokines (Widlansky et al., 2003). The endothelium's ability to vasodilate is reduced, and ultimately this environment may contribute to the development and clinical expression of atherosclerosis (Widlansky et al., 2003). "Endothelial function appears to participate in a 'positive feedback loop' in which inflammatory factors promote monocyte and T-cell adhesion, foam cell formation, extracellular matrix digestion, and vascular smooth muscle migration and proliferation leading to atherosclerotic plaque formation" (Widlansky et al., 2003).

Understanding the consequences of endothelial dysfunction as it relates to atherosclerosis is important in developing therapeutic strategies such as diet to improve endothelial function.

When unable to vasodilate properly, the endothelium remains in a normotensive or constricted state, resulting in endothelial dysfunction. Current research indicates that a connection between endothelial dysfunction, as indicated by impaired EDD occurring over a span of time, and HTN exists (Taddei et al., 1995; Panza et al., 1990; Widlansky et al., 2003; Bonetti et al., 2003). While some studies have found that EDD is impaired in HTN, it is not likely to be the root cause of HTN (Mombouli and Vanhoutte, 1999). This may be due to the fact that endothelium-derived constricting factor is also found in high concentrations in normotensive individuals with a family history of HTN (Taddei et al., 1995). However, minimizing endothelial dysfunction may be important in slowing or preventing the development of future HTN.

1.5 Sodium and Potassium

1.5.1. Recommended Intakes

The standard guidelines for the recommended intake of sodium and potassium intake come from the Dietary Guidelines for Americans (DGA, 2010). These guidelines are updated and published every five years. The most recent one, the 2010 DGA recommend lowering sodium intake from the current average intake of 3,400 mg/d to <2,300 mg/d or 1,500 mg/d depending on age,

race/ethnicity, and presence of chronic disease. In regards to age, after the age of 51, the recommended sodium intake is no more than 1,500 mg/d (CDC, 2010). Data from the NHANES 2008 shows that across each age group, less than 1% of Americans are consuming <1,500 mg/day, with nearly the lowest percentage of optimal consumption occurring in those 60 years and older.

The DGA recommends a potassium intake of 4,700 mg/d for men and women, and these recommendations do not change between age groups; however, there is a more urgent recommendation to consume the recommended amount of potassium after the age of 51 due to its protective benefits for the vasculature (National Academy of Sciences, 2010; CDC, 2010). Actual potassium intake is much lower than the recommended value. After age 20, men consume an average of 3,172 mg/d, while women consume an average of 2,640 mg/d (What We Eat in America; Potassium Intake, 2010). Further, commercially prepared foods are low in potassium contributing to our historically low intakes. Studying sodium and potassium's effects on blood pressure is imperative due to the increasing trend of higher sodium and lower potassium consumption than recommended levels in the United States (What We Eat in America, 2010).

1.5.2. Dietary Studies of Sodium and Potassium Intake.

Sodium consumption in the US diet has increased over time (CDC, 2009). This increase in sodium content in the diet may be due to several factors. First, from 1971 to 2004, total energy consumption among US adults increased by 22% in women and 10% in men (NHANES, 2008). This increase in total energy

intake is partially attributable to increased consumption of sodium-laden snacks and commercially prepared food (He et al., 1999). Research has consistently shown that high salt diets may lead to increased incidence of HTN, and thereby increased mortality rates (Appel et al., 2006; Danaei et al., 2009; Cook et al., 2007; Cook et al., 2009; Bruce et al., 1990; Umesawa et al., 2008). In response to this, the Dietary Approaches to Stop Hypertension (DASH) diet trials were undertaken to evaluate the role of diet in lowering blood pressure. The DASH diet substantially lowers blood pressure in hypertensive and non-hypertensive populations by emphasizing a low sodium and high potassium diet. This diet consists of ample fruits and vegetables, low-fat dairy products, whole grains, poultry, fish, and nuts, as well as a reduction in total fat, saturated fat, and cholesterol (Appel et al., 1997). When comparing an intervention diet consisting of high (3,400 mg), medium (2,300 mg), and low (1,150 mg) salt consumption in combination with either a diet typical of the American diet or the DASH diet, researchers found that a low-salt diet combined with the DASH diet proved to be the most effective dietary combination, reducing SBP by an average of 7.1 mmHg in hypertensives (Sacks et al., 2001).

A large-scale meta-analysis was conducted on blood pressure changes in 40 trials evaluating sodium reduction and 27 trials evaluating increased potassium (Geleijnse et al., 2003). They found that “sodium reduction (median: -1,771 mg/d) was associated with a change of -2.54 mmHg in SBP and -1.96 mmHg in diastolic blood pressure. Corresponding values for increased potassium

intake (median: 1,716 mg/d) were -2.42mmHg and -1.57mmHg" (Geleijnse et al., 2003). In addition, they found that the blood pressure response was greater in hypertensives than normotensives, both for sodium (systolic: -5.24 versus -1.26 mmHg, $p < 0.001$; diastolic: -3.69 versus -1.14 mmHg, $p < 0.001$) and potassium. Therefore, blood pressure is affected by sodium and potassium intake in normotensive individuals, but not as drastically as hypertensive individuals. Similarly, in regards to aging, blood pressure decreased significantly more in the older population than the younger population when sodium and potassium intake was altered. In total, a low sodium and high potassium diet is beneficial for lowering blood pressure, particularly in an older population.

Chronic salt loading has been shown to alter blood pressure. In a study by MacGregor et al (1989), after subjecting mildly hypertensive subjects to a reduced sodium diet of 1,150 mg/d for one month, researchers initiated a 3-month study of 3 levels of sodium intake (4,600, 2,300, and 1,150 mg/d). Systolic blood pressure was significantly reduced in the moderate and low-intake sodium levels with an average reduction of 16 and 9 mmHg. In a study by Pimenta et al (2009), researchers randomly placed two groups of hypertensive participants currently on an antihypertensive medication regimen in 1 of 2 controlled diet groups consisting of a low sodium (1,150 mg/d) or high sodium (>5,750 mg/d) diet. After the initial one-week of the controlled diet, subjects resumed their normal diet for two weeks, then crossed over to the opposite diet for one week. Researchers found that in patients with resistant HTN, reducing

sodium intake by 4,600 mg/d lowered SBP and DBP by 22.7 and 9.1 mmHg, respectively. Likewise, they found that an increased sodium diet suppressed the effect of antihypertensive medications. In regards to urinary excretion of sodium, one meta-analysis concluded that a modest reduction in sodium intake for four or more weeks reduced urinary sodium excretion by 78 mmol/d (equivalent to 4.6 grams of salt per day), with a concomitant decrease in SBP and DBP of 2.0 and 1.0 mmHg in normotensive individuals and 5.0 and 2.7 mmHg in hypertensive individuals (He and MacGregor, 2002). In summary, there is a general consensus that acute and chronic sodium loading negatively affects the vasculature and blood pressure, and sodium reduction improves these effects.

1.5.3. Excretion of Sodium and Potassium

The urinary excretion of sodium and potassium has been used in several large-scale human studies to evaluate cardiovascular disease risk (Scott et al., 2011; Adrogue and Madias, 2007; Cook et al., 2009; Geleijnse et al., 2007). These electrolytes are expressed as a ratio (sodium:potassium excretion ratio). This ratio is determined from the collection of urine over a 24-hour period, followed by electrolyte level analysis of sodium and potassium. The levels of sodium and potassium in the urine are adequate reflections of dietary intake as these electrolytes are tightly controlled by the kidney (Guyton, 1981). The kidney is constantly maintaining a homeostatic environment, as there are constant undulations in electrolyte concentrations. The environment in the cell cytosol is high in potassium and low in sodium, whereas in the extracellular space

potassium concentration is low and sodium concentration is high (Guyton, 1981). This concept is important because driving forces of the electrolyte gradient provide the basis for cell function and many cell functions are very sensitive to changes in the extracellular fluid potassium concentration (Guyton, 1981). Postprandially, the extracellular and intracellular levels of these electrolytes change. These changes occur in the nephron of the kidney, which reabsorbs and filters water and electrolytes in response to dietary intake (Guyton, 1981). Electrolyte excretion is determined by the sum of three processes in the kidney: electrolyte filtration rate, the rate of electrolyte reabsorption by the tubules, and the rate of secretion of electrolytes by the tubules (Guyton, 1981).

There are many determinants of sodium filtration. Two of the main systems involved in the control of renal sodium and water excretion are the RAAS and renal sympathetic nerves (Guyton, 1981). The RAAS is initiated if there is a decrease in glomerular filtration rate, due to a decrease in arterial pressure and glomerular hydrostatic pressure. The macula densa senses the change in solute concentration (Guyton, 1981). If there is low sodium and/or high potassium intake, the RAAS is triggered, whereby renin, angiotensin II, and efferent arteriolar resistance are increased (Guyton, 1981). Glomerular hydrostatic pressure and thereby glomerular filtration rate are increased in a negative feedback mechanism (Guyton, 1981). In the rest of the nephron, water and electrolytes are reabsorbed. In the proximal tubule, 65% of the water and electrolytes are reabsorbed. Sodium is also reabsorbed in the thin ascending

loop of Henle, thick ascending loop of Henle, distal tubule, and medullary collecting tubule (Guyton, 1981). Water is reabsorbed in the thin descending loop of Henle (Guyton, 1981). The RAAS decreases sodium excretion in response to decreased effective circulating volume and vice versa (Guyton, 1981). Renin release is also triggered by sympathetic activation due to baroreceptor loss of signaling (Guyton, 1981). Baroreceptors sense blood pressure changes. An increase in nerve activity decreases sodium and chloride excretion.

Regulation of potassium balance depends primarily on excretion by the kidneys. The proximal tubule and the ascending loop of Henle are responsible for potassium reabsorption, while the distal tubule and cortical collecting duct are the key portions of the nephron which control potassium excretion and secretion, by way of the sodium-potassium ATPase pumps in the principal cells (Guyton, 1981). Uptake of potassium into cells is affected by insulin, epinephrine and aldosterone (Guyton, 1981). Insulin is the most important hormone that shifts potassium into cells after a meal (Guyton, 1981). Likewise, the binding of epinephrine to beta-2 adrenergic receptors is essential for uptake of potassium into cells after a meal (Guyton, 1981). Lastly, aldosterone increases with greater potassium consumption, thereby increasing potassium uptake in cells, and also regulating secretion of potassium by increasing the permeability of the luminal membrane for potassium (Guyton, 1981). After a meal, potassium is quickly transported into the cell because even slight changes in the extracellular fluid can

be toxic (Guyton, 1981). The kidney is then responsible for eliminating the excess.

The sodium:potassium excretion ratio has become an important marker of increased CVD risk. Many large-scale studies have utilized the ratio to quantify sodium and potassium intake and determine mortality risk (Scott et al., 2011; Adrogue and Madias, 2007; INTERSALT, 1988). INTERSALT (1988) studied the relationship between 24-hour urinary electrolyte excretion and blood pressure in 10,079 men and women aged 20-59 sampled from 52 countries. Researchers found that the sodium:potassium excretion ratio significantly correlated with SBP and DBP. Interestingly, in the four countries whose populations had very low sodium excretion (0.2 – 51.3 mmol/d) and low urinary sodium:potassium excretion ratios (<0.01 – 1.78) median blood pressures were also low (SBP 95 - 110 mmHg, DBP 61 - 68 mmHg) and the slopes of blood pressure with age were negative or small and positive. The other countries analyzed had positive blood pressure slopes with age and higher median blood pressures.

1.5.4. Sodium's Effects on the Vasculature

Sodium consumption can have a profound effect on the vasculature as shown by both human and animal studies (Bragulat et al., 2001; Tzemos et al., 2008; Nurkiewicz and Boegehold, 2007; Zhu et al. 2004). There are several proposed mechanisms by which increased sodium affects endothelial function. One, increased sodium intake can decrease NO production and/or bioavailability, as shown in a human hypertension model (Fujiwara et al., 2000). In this study, in

the presence of high salt, a decrease in synthesis of NO accompanies an increase in plasma ADMA, an inhibitor of NO production (Fujiwara et al., 2000). Two, an increase in salt concentration significantly suppresses eNOS activity, also affecting NO bioavailability (Li et al., 2009). eNOS catalyzes the production of NO from L-arginine when NADPH donates electrons which react with the BH_4 and generate citrulline and NO as byproducts (Alderton and Cooper, 2005). Three, high salt diets have been shown to contribute to oxidative stress, thereby affecting vasodilation (Annuk et al., 2001; Heitzer et al., 2001). Specifically, by increasing plasma ADMA levels, eNOS is uncoupled and produces superoxide, a reactive oxygen species (ROS) that is highly reactive with other molecules (Fujiwara et al,. 2000). Superoxide can then oxidize other molecules causing further damage. An increase in oxidative stress may compromise endothelial and vascular function, leading to endothelial dysfunction. In patients with HTN, the extent of oxidative stress is highly correlated with the level of impairment of endothelium dependent dilation (EDD) (Annuk et al., 2001; Heitzer et al., 2001). In total, as oxidative stress levels increase, so does the impairment of EDD as well as the increased risk of cardiovascular events due to dilatory impairments of the endothelium.

Another potential mechanism by which increased sodium consumption compromises the vasculature is alterations in cell structure and shape. Spindle-shaped endothelial cells have decreased NO production and increased stiffness related to dense actin stress fibers, while large, circular endothelial cells have

increased NO production and are soft, lacking dense actin stress fibers (Hutcheson and Griffith, 1996). Due to NO's ability to depolymerize actin (decreasing stiffness), it is plausible that elastic properties are determined by NO bioavailability (Hutcheson and Griffith, 1996). Elasticity of the endothelial cells was also examined by Peng et al., (2003) as researchers compromised endothelial cell elasticity by placing them in a rigid tube and subjecting them to pulsatile flow. Without normal elasticity, eNOS production was markedly decreased. If high sodium intake is present and subsequent NO bioavailability is low due to a decrease of eNOS, there will presumably be morphological changes in the cellular structure of the endothelium, as sodium prevents the swelling and/or softening of endothelial cells, decreasing NO (Oberleithner et al., 2009). Therefore, a high sodium diet could cause morphological changes in endothelial cell structure resulting in subsequent endothelial dysfunction.

The effect of a high sodium intake on vascular function has been studied. In an acute study, in young, healthy females, Cavka et al (2011) found that one week of a low salt diet (<920 mg/d) followed by one week of a high salt diet (>4,600 mg/d) caused impaired peripheral blood flow of the brachial artery when measuring blood flow by laser Doppler after an occlusion protocol. Tzemos et al (2008) also observed impaired EDD in young, healthy men after acute sodium loading (4,600 mg/d for 5 days). While these two studies examined the effect of a high salt diet for one week, another study of healthy adults examined the immediate postprandial effects of a high salt diet versus a low salt diet. They

found impaired brachial artery FMD just 30 minutes after consumption of a high salt meal (Dickinson et al., 2011). In summary, a high sodium diet has acute effects on the vasculature.

1.5.5. Potassium's Effects on the Vasculature

An increased potassium intake has been shown to positively affect the vasculature by decreasing blood pressure. This is thought to occur by hyperpolarization of the vascular smooth muscle cells, allowing them to relax and vasodilate (Sobey, 2001). This hyperpolarization is subsequent to potassium's stimulation of the sodium/potassium pump and activation of the plasma membrane potassium channels (Haddy et al., 2006; Amberg et al., 2003), and has been shown in studies where extracellular potassium increases and extracellular sodium decreases (Edwards et al., 1998; Dawes et al., 2002; Bussemaker et al., 2002). Potassium has many protective benefits in addition to decreasing blood pressure. They include lowering the risk of stroke (D'Elia et al., 2011; Khaw and Barrett-Connor, 1987), preventing the development of kidney damage by improving kidney functioning (Mattheson et al., 2012; Tobian et al., 1984), reducing formation of kidney stones (Ettinger et al., 1997), reducing osteoporosis (Zhu et al., 2009), and protecting the vasculature in HTN by lowering ROS (Kido et al., 2008). Potassium ions also contribute to endothelium-dependent relaxation in that they are released by the endothelial cells in response to physical forces such as shear stress (Haddy et al., 2006). Likewise, in a study in bovine aortic endothelial cells, an acute increase in potassium was

shown to swell and soften the endothelial cells thereby increasing NO release (Oberleithner et al., 2009). Therefore, an increase in potassium intake can improve the vasodilatory properties of the vasculature and counteract the harmful effects of sodium.

While a high potassium intake appears protective, a low potassium intake can negatively affect blood pressure and the vasculature. Many studies have shown a marked increase in blood pressure with low dietary potassium intake (van Mierlo et al., 2010; Dolson et al., 1995; Geleijnse et al., 2003; INTERSALT, 1988). When looking at a low potassium intake independent of sodium, a depletion of potassium dramatically increases blood pressure in hypertensive and normotensive populations (Krishna et al., 1989, 1991; van Mierlo et al., 2010; Whelton et al., 1997; Sica et al., 2002). The INTERSALT (1988) study found that a decrease in potassium excretion by 50 mmol/d increased SBP 3.4 mmHg and increased DBP 1.9 mmHg. In a meta-analysis by van Mierlo et al (2010), researchers used population-based surveys from 1990 to 2009 in which potassium intake was surveyed from more than 1,000 adults across the world. In addition, they collected blood pressure data for Finland, the United Kingdom, and the United States, which represented high, medium, and low potassium intakes, respectively. With these data, researchers were able to predict the impact of increasing potassium intake on lowering blood pressure. They found that average potassium intakes ranged from 1,700 mg/d (China) to 3,700 mg/d (Finland, The Netherlands, Poland). They were able to calculate that a

hypothetical increase in potassium intake to 4,700 mg/d would lower SBP 1.7 to 3.2 mmHg in Western countries. This increase in potassium would also increase the number of people in the optimal SBP category (<120 mmHg) by 2-5% and 4-8% for men and women, respectively.

1.5.6. Sodium and Potassium and Vascular Health

The sodium:potassium urinary excretion ratio has become an increasingly important marker of CVD risk as recent studies have shown that the ratio is significantly correlated with SBP and an increased risk of CVD (Cook et al., 2009; Scott et al., 2011; Adrogue and Madias, 2007). It is important to note that when sodium excretion or potassium excretion was studied alone, neither showed a significant effect in the above studies. However, this finding is not consistent. Geleijnse et al (2007) found no consistent association between the ratio, sodium excretion or potassium excretion with CVD or all-cause mortality in their randomly-selected population of older adults (over 55 years old). The only relationship they could establish was between the ratio and all-cause mortality in the overweight subjects. The lack of positive correlation in all subjects may have been due to the small range of sodium intake in the subject pool (795 subjects from the Netherlands). Risk of CVD and the sodium:potassium ratio was also assessed in a meta-analysis by Yang et al (2011) using NHANES III data, a nationally representative sample of the U.S. population of all age groups. They concluded that a higher sodium:potassium excretion ratio was associated with a significant increase in risk of CVD, due to its effect on blood pressure. In

conclusion, most studies have shown that the sodium:potassium excretion ratio is a good marker to use in a large population when assessing the risk of CVD due to its relevance and efficacy, as opposed to assessing sodium or potassium alone. What has not been evaluated is whether the sodium:potassium excretion ratio correlated with vascular function.

1.6 Aging and Vascular Health

The prevalence of HTN increases dramatically with age. Approximately 90% of older adults will eventually become hypertensive (Vasan et al., 2002). Prevalence of HTN is 8 times greater in adults aged 75 or older compared to their younger counterparts aged 20-34 (NHANES, 2008). Not only is there an increased risk of HTN with aging, but there is also an increased incidence of impaired vascular function. Older adults have stiffer vessels (Jablonski et al., 2009; Seals et al., 2011). Research shows that there is a consistent correlation between SBP and diminished EDD in middle-aged to older adults when subjects are age-, and sex-matched with normotensive counterparts (Taddei et al., 1997; Wang et al., 1999). Also, unpublished data from Jablonski et al., as published in Seals et al (2011), showed that older adults with essential HTN have lower FMD compared to normotensive young and normotensive older adults. Similar results are seen in animal studies. In an aging rodent model, endothelium-dependent dilators were drastically reduced, resulting in a further impairment of the endothelium to produce NO (Cernadas et al., 1998; Matz et al., 2000). There is

also an impaired endothelium-dependent hypotensive response to vasodilators, believed to be the result of decreased activity of eNOS (Cernadas et al., 1998). Also in aging rodent arteries, endothelium-dependent hyperpolarization is significantly reduced compared to young rats (Nakashime and Vanhoutte, 1993). This impairment is due to a decreased release of endothelium-dependent hyperpolarizing factor, leading to vasoconstriction (Nakashime and Vanhoutte, 1993). In summary, the prevalence of HTN increases with age and there are many potential mechanisms by which the age-related increase in blood pressure vascular function.

Sodium and potassium intake therefore need to be monitored more closely with an aging population as their vascular health progressively decreases with age as arteries become stiffer (Jablonski et al., 2009; Seals et al., 2011; Cernadas et al., 1998). Not only does HTN and vascular dysfunction increase with age (Roger et al., 2012; Kearney et al., 2005; Seals et al., 2011), but blood pressure sensitivity to sodium has been shown to increase in healthy older adults (Elliot et al., 1996; Zemel and Sowers, 1988). Therefore, in aged population, sodium intake is likely to have a greater impact on vascular health, compared to younger adults. A study in post-menopausal women found that both sodium reduction and exercise improved vascular health; however, the reductions in SBP and pulse pressure due to sodium reduction were 3 to 4 times greater than the exercise intervention (Seals et al 2001). Although these researchers did not evaluate the sodium:potassium excretion ratio, they concluded that there is a

potential mechanistic role in the hypotensive effects of sodium reduction, due to the reductions in central arterial stiffness. Likewise, low sodium consumption (as determined by self-reported dietary intake) was related to enhanced brachial artery FMD in both mature and older adults (Jablonski et al., 2009). Bray et al (2004) found that sodium reduction to 1,500 mg/d can more drastically lower SBP in aged adults compared to young adults. In the 55-76 year old group, researchers found an 8.1 mmHg decrease in SBP compared to a 4.8 mmHg decrease in the 23-41 year old group. In regards to potassium, following the DASH diet or diets with a similar design has been shown to improve vascular function. FMD has been shown to improve in older adults with moderately elevated SBP when following the DASH diet (Blumenthal et al., 2010). Overall, research has consistently shown that in older adults, decreasing sodium and increasing potassium intake has positive benefits on blood pressure and vascular health (Jablonski et al., 2009; Appel et al., 2006; Danaei et al., 2009).

1.7 Specific Aim and Hypothesis

Hypertension (HTN) is a significant Public Health problem and the risk of an elevated blood pressure increases drastically with age. Likewise, dietary factors can influence the development of HTN and the effects of a high sodium/low potassium diet on increasing blood pressure are well-known (INTERSALT, 1988; Cook et al., 2007 and 2009; Sica et al., 2002; Whelton et al., 1997; Trials of Hypertension, 1997). Research has shown that reducing sodium

intake can decrease blood pressure and the incidence of cardiovascular disease (INTERSALT, 1988; Appel et al., 2006; Danaei et al., 2009; Jablonski et al., 2009; Seals et al., 2001). Further, it has been shown that a high potassium diet can decrease blood pressure and therefore, decrease the incidence of CVD (van Mierlo et al., 2010; Dolson et al., 1995; Geleijnse et al., 2003; INTERSALT, 1988; D'Elia et al., 2011). While these two electrolytes have been studied extensively in relation to HTN, it is becoming clearer that they may impact the vasculature prior to a change in blood pressure (Jablonski et al., 2009; Seals et al., 2011). Indeed, there have been multiple studies on sodium's negative effects on the vasculature (Bragulat et al., 2001; Tzemos et al., 2008; Nurkiewicz and Boegehold, 2007; Zhu et al. 2004), while significantly less studies have been reported on potassium. Given the important role each electrolyte plays on the vasculature, more recent evidence suggests that the interaction between sodium and potassium may be more important than their independent effects on blood pressure and CVD mortality (Cook et al., 2009; Scott et al., 2011; Adrogue and Madias, 2007). To date, there have only been a minute amount of studies examining the effects of a high sodium/low potassium diet on vascular function (Seals et al., 2001; Jablonski et al., 2009; Bray et al., 2004; He et al., 2001; Blumenthal et al., 2010; Park et al., 2011; Polonia et al., 2006). Further, the number of published studies in older populations is limited and these studies have not evaluated the urinary sodium:potassium excretion ratio (Seals et al., 2001; Jablonski et al., 2009; Bray et al., 2004; He et al., 2001; Blumenthal et al.,

2010), an increasingly important predictor of cardiovascular events and mortality (Cook et al., 2009; Geleijnse et al., 2007; Langford, 1991). The proposed study is unique in that it will study the role of habitual dietary intake of sodium and potassium on vascular health in healthy, older adults using the urinary sodium:potassium excretion ratio as a marker of vascular health. Therefore, the following aim has been proposed:

Specific Aim

The specific aim of this study was to examine the effect of habitual sodium and potassium intake on vascular function in a healthy, older adult population.

Hypothesis

We hypothesize that a high sodium/low potassium intake resulting in a higher sodium:potassium excretion ratio will be associated with poorer vascular function in healthy, older adults while a low sodium/high potassium intake resulting in a lower sodium:potassium excretion ratio will correlate with better vascular function.

Chapter 2

METHODS

2.1 Subjects

All protocols and procedures were approved by the University of Delaware Institutional Review Board. Written consent was obtained from all subjects. Thirty subjects (16 male, 14 female) were recruited for the study. Subjects averaged 62 ± 1 years old and were apparently healthy. Subjects were recruited from the university and surrounding community via advertisements (Appendix A). Subjects were normotensive or prehypertensive (<140/90 mmHg).

2.1.1. Exclusion Criteria

Subjects were excluded for diabetes, kidney disease, high blood pressure, coronary heart disease, stroke, arrhythmias, peripheral vascular disease, obesity (as defined by a BMI ≥ 30), and lung disease as assessed by medical history questionnaire.

2.2 Visit 1: Screening Visit

A flow chart progression of the timeline of events is shown in Figure 1. Testing took place in the Vascular Physiology Laboratory in McDowell Hall at the University of Delaware.

During the screening, the informed consent was read, reviewed, and signed (Appendix D), and a medical history questionnaire from the Nurse Managed Health Center (Appendix B) was completed. Resting brachial blood pressure (Dinamap Dash 2000; GE Medical Systems, Milwaukee, WI) was measured 3 times and averaged. Subjects were excluded for participation if their SBP was ≥ 140 mmHg and/or their DBP was ≥ 90 mmHg. Subjects' height and weight were measured and recorded. Subjects were given a packet of instructions for recording their diet (Appendix C). They were also given an Acti*Cal* (Acti*Cal*, Koninkljke Philips Electronics, Andover, MA), calibrated to the subject's height, weight, age, and sex, and they were instructed on how to use the device (Appendix F). An Acti*Cal* is a small device that measures active energy expenditure. Lastly, they were given a backpack with a 3 liter urine collection container to collect 24 hours of urine, and they were instructed on collection procedures (Appendix E).

2.3 Collection Procedures

Subjects were instructed to record their dietary intake of food and beverage for 24 hours on two weekdays and one weekend day. They were given a document which included detailed directions, sample portion sizes, a 3 day food record, and a dietary supplement recording sheet. The subject used his or her discretion to decide which day and the order of days to collect their data; however, the third day had to have preceded their second visit to the laboratory.

During these three days, subjects were instructed to wear the Acti*Cal* around their hips, positioned directly inside the right hip bone. They were not instructed to wear the device in the shower, in water, or to sleep. They were given the option to press the star button on the Acti*Cal* upon beginning exercise. This button placed a tick mark in the recording for the researcher's convenience. Each day that the Acti*Cal* was not utilized, subjects were instructed to keep the device in a location void of movement. The subject was also asked a series of questions pertaining to the time and intensity of exercise, physical activity, sleep, and work that they engaged in throughout the week (Appendix G). This data provided a back-up if the Acti*Cal* malfunctioned.

On the last day of self-collection, the subject was instructed to collect 24 hours of urine. Upon waking on the last day of collection, the first urine deposit was not collected because that deposit was remnants from the prior day. After that, though, subjects began collection for 24 hours, recording the time of their first and last deposits. Upon waking on the day of the assessment, the subject collected the first deposit because that deposit was remnants from the previous day of collection. After that, subjects visited the lab for final assessments.

2.4 Visit 2: Vascular Function Testing and Venous and Urine Sampling

The second visit to the lab consisted of vascular function testing and blood and urine sampling. Subjects were asked to refrain from exercise for 24 hours prior, alcohol and caffeine for 12 hours before, and food for 4 hours prior to the

final visit to the lab. The subject was connected to a 3-lead EKG for monitoring of heart rate throughout the protocol. Resting blood pressure was also taken and averaged from 3 recordings. PWA was then performed, measuring the degree of wave reflection propagation. PWA was followed by PWV, which recorded pressure readings of the main arteries in the groin and neck. A cloth tape was used to measure the distance from the carotid transducer to the sternal notch, sternal notch to the navel, and navel to the femoral artery transducer in order to calculate velocity. FMD of the brachial artery was the last measurement.

2.4.1. Blood and Urine Analysis

Phlebotomy was performed by trained personnel in the Nurse Managed Health Center by inserting a needle into the antecubital space (approximately 3 tablespoons or 45 mL of blood was removed). The blood collected was transferred to the appropriate Vacutainer tube and spun for 15 minutes at 2,500 rpm in an Eppendorf 5810 Centrifuge (Hamburg, Germany). All measurements were made in triplicate, performed after quality control standards were run. Blood was drawn for immediate analysis of hemoglobin, hematocrit, plasma electrolytes sodium, potassium, and chloride, and osmolality. Whole blood was transferred to precalibrated capillary tubes and spun in triplicate on a Readacrit Centrifuge (Clay Adams Brand, Becton Dickinson, Parsippany, NJ) to determine hematocrit. Whole blood was transferred to collecting slides for the analysis of hemoglobin (Hemocue Hb 201 Analyzer, Hemocue, Lake Forest, CA). The

serum potassium, sodium, and chloride and plasma osmolality were determined (EasyElectrolyte Analyzer, Medica, Bedford Bedford, MA; Model 3D3 Osmometer, Advanced Instruments, Norwood, MA). Excess blood was spun separately and stored at -80° C.

Urine sodium, potassium, and chloride and plasma osmolality were determined (EasyElectrolyte Analyzer, Medica, Bedford Bedford, MA; Model 3D3 Osmometer, Advanced Instruments, Norwood, MA). All urine measurements were made in quintuplicate, performed after quality control standards were run. Specific gravity was also measured (Goldberg TS Meter Clinical Refractometer, Reichert Technologies, Depew, NY). Urine was also stored for future use at -80° C.

2.4.2. Pulse Wave Analysis

To measure pulse waves for calculation of PWA, a pencil-type probe, or tonometer (Millar Instruments, Houston, TX) was placed over the radial artery while the subject was in a supine position. The tonometer was connected to the SphygmoCor Px System (AtCor Medical, Sydney, Australia) and the radial wave was recorded. A central pressure wave was calculated non-invasively from the arterial pressure wave via a transfer function. This PWA technique and subsequent transfer function have been validated in various studies (Chen et al., 1997; Gallagher et al., 2004). The augmentation index (AIx) was described as the approximate amplitude of the central systolic arterial wave reflection. AIx

was recorded as (Ps-Pi)/(Ps-Pd), where Ps = peak systolic pressure, Pi = the inflection point occurring in systole either before or after peak systolic pressure, and Pd = diastolic pressure. A pressure wave was accepted if two criteria were met: signal strength of greater than 300 and an operator index of over 80 (reproducibility). The best 3 waveforms were then averaged, and AIx was recorded.

2.4.3. Pulse Wave Velocity

Carotid-femoral PWV was calculated by measuring the propagation time of the pulse pressure wave from the difference between the average of 12 time durations from the R-wave of an EKG waveform to the initial upstrokes of the waveforms from the carotid and femoral arteries. In a supine position, a pressure transducer (Millar Instruments, Houston, TX) was placed on the femoral artery. When a sufficient waveform was read, the signal was recorded for 30 seconds. The transducer was then re placed on the carotid artery. The artery waveforms were recorded for 30 seconds. Landmark to landmark was physically measured with a cloth tape measure from carotid transducer to sternal notch, sternal notch to umbilicus, and umbilicus to femoral artery transducer. Carotid-femoral PWV was calculated as the combined distance from suprasternal notch to the umbilicus and from the umbilicus to femoral site minus carotid arterial length ($PWV_{([ssn–umb]+[umb–fem])-(car-ssn)}$). PWV was then calculated by the difference between the average measured time delay between the initial upstrokes of twelve consecutive R-waves (in the EKG reading) and corresponding femoral

waveforms, and the average measured time delay between the initial upstrokes of twelve consecutive R-waves and corresponding carotid waveforms. This average time was divided into the length calculated (units were in cm/s). Carotid-femoral PWV is a regional measurement of arterial stiffness.

2.4.4. Brachial Artery Flow-Mediated Dilation

Measurement of endothelium-dependent FMD of the brachial artery was performed using high resolution ultrasound (Tital, Sonosite Inc.) equipped with an EKG following published guidelines (Corretti et al., 2002). An inflation cuff was placed on the forearm immediately distal to the antecubital space. Brachial artery vasodilatory responses to hyperemia were measured in the brachial artery of the non-dominant arm using a 10 MHz linear phased array ultrasound transducer (Logiq e, GE Healthcare, Waukesha, WI) to produce longitudinal images of the brachial artery.

When a sufficient image of the brachial artery was found, baseline images and blood velocity were obtained and recorded for the baseline measurement of end diastolic diameter. Next, the cuff was inflated to 200 mmHg for 5 minutes. Brachial artery images and blood velocity images were recorded from 30 seconds prior to inflation to 2 minutes after deflation (LabVIEW 10.0) to determine percent dilation and peak diameter change due to reactive hyperemia. Ultrasound images were transmitted to a National Instruments IMAQ PCI-1411 image acquisition board by way of an S-Video connection at a frequency of 20 frames per second.

Brachial artery diameter was determined using custom designed automated edge detection software in National Instruments LabVIEW 8.6. Percent dilation was measured manually. A section of the image was selected by drawing a box around the section. Quality of the image was verified by manually viewing the selected portion at 500 frame intervals. The placement and contrast threshold was adjusted accordingly. When a sufficient quality section was determined, the image was analyzed by the program. Peak diameter was determined after applying a 3-second-wide median filter to each data point. FMD was expressed as a percent increase in diameter from baseline (change in diameter divided by baseline diameter).

2.4.5. Dietary Analysis

Dietary intake was assessed using the Nutrient Data Systems software for Research (Minneapolis, Minnesota, USA). The 3 days of intake were averaged to get an estimate of each subject's typical nutritional intake.

2.5 Statistical Analysis

The sodium:potassium urinary excretion variable was the main variable of concern and the primary goal was to determine if there was an inverse relationship between this ratio and measurements of vascular function. Data with a linear distribution was tested using Pearson correlations. Data with a nonlinear distribution was tested using the Spearman correlation. Subjects were also separated into high and low sodium intake, potassium intake, sodium excretion,

potassium excretion, and sodium:potassium excretion ratio based upon median values of the respective values. Two-tailed unpaired t-tests were used to compare high and low groups. Statistical significance was set at $p < 0.05$ for all analyses (Microsoft Excel, Version 2010).

Sodium intake was normalized to total energy intake. Physical activity was used as a covariate for FMD and the sodium:potassium excretion ratio and energy intake was used as a covariate for sodium intake and sodium excretion. Lastly, HR was held constant while looking at the relationship between sodium excretion and ED, and the sodium:potassium excretion ratio and ED. Statistical significance was set at $p < 0.05$ for all analyses (SPSS, Version 19.0).

Chapter 3

RESULTS

3.1 Subject Characteristics

Subject characteristics are presented in Table 1. A total of 16 males and 14 females participated in the study. All subjects were normotensive with an average blood pressure of 120/72 mmHg. The average BMI was 26.6 ± 0.7, putting subjects in the overweight category. When comparing the males and females, there were no significant differences in age, BMI, SBP, DBP, HR, or total energy expenditure (all $p > 0.05$). As expected, there were differences in height ($p < 0.001$), body mass ($p < 0.01$), hemoglobin ($p < 0.05$) and hematocrit ($p < 0.05$) between the males and females.

3.2 Blood and Urine Values

Blood and urine values are shown in Table 2. There were no significant differences in serum sodium (Na+), serum potassium (K+), plasma osmolality, 24-hour urinary K+ excretion, the urinary Na+:K+ excretion ratio, urine volume or urine flow rate between the males and females (all $p > 0.05$). However, there was a significant difference in 24-hour urinary Na+ excretion ($p < 0.001$), urine osmolality ($p < 0.05$), and urine specific gravity ($p < 0.05$).

3.3 Diet Composition

Habitual dietary intake of the subjects is shown in Table 3. Total energy consumption, as well as the percentage of carbohydrate, fat, and protein relative to energy intake was not different between males and females however, although not statistically significant, the females consumed more fat, and less carbohydrate and protein as a percentage of their total energy. The recommended acceptable macronutrient distribution range for fat is 20-35% and the females slightly exceeded this at 36.5%. The intake of the major minerals including sodium, potassium, calcium, phosphorus, and magnesium was not significantly different between males and females (all $p > 0.05$), nor was consumption of alcohol or caffeine ($p>0.05$). Additional vitamins are shown in Table 4. There were no significant differences between males and females in regards to vitamin D, vitamin C, vitamin E, or phosphorous intake. Six of the 30 subjects took a multivitamin. The micronutrient intake from the multivitamin did not signifciantly altered their intakes. There was a significant difference in vitamin B6 consumption between males and females ($p < 0.01$).

For the two key nutrients of interest, average sodium intake was 3,187 $\pm$ 169 (with a range of 2,077 – 6,413 mg) and 2,937 $\pm$ 219 mg (with a range of 1,921 – 4,167 mg) for males and females, respectively. This level of intake is above the recommendation from the 2010 Dietary Guidelines for Americans.

Average potassium intake was 3,307 ± 256 (1,461 – 5,169 mg) and 2,907 ± 229 (1,824 – 5,587 mg) mg for males and females, respectively and this fell below the recommendations from the 2010 Dietary Guidelines for Americans. The relationship between sodium intake and potassium intake in males and females is depicted in Figure 2. Upon normalizing sodium to total energy intake, the only significant relationship found was with ED ($r = -0.419$, $p < 0.05$). No other relationship with vascular measurements existed. To verify that subjects accurately recorded their dietary intake, correlations were run between sodium and potassium intake and the urinary excretion of these nutrients. Indeed, sodium intake did correlate with sodium excretion (see Figure 4; $p < 0.05$) and potassium intake correlated with potassium excretion (see Figure 5; $p < 0.05$) suggesting that subjects did report everything they consumed. Using energy as a covariate in the relationship between sodium intake and excretion, no significance was found ($r = 0.2$, $p = 0.298$). Further, while the intake of sodium and potassium was higher in males than females, the intake ratio between these two nutrients was identical between the sexes (1.1 ± 0.09 and 1.1 ± 0.09 for males and females, respectively). Again, the ideal intake ratio of 0.49 would reflect a diet containing 2,300 mg of sodium and 4,700 mg of potassium.

3.4 Vascular Measurements

Vascular measurement values are shown in Table 5. HR-adjusted AIx was significantly greater in women (19 ± 2.5 versus 26 ± 2.1%, $p < 0.05$);

however, this is expected given that the women are generally shorter than men, which is reflected in the current data. AIx (26 ± 2.8 versus 33 ± 2.4%, p = 0.059) and ejection duration (335 ± 4.6 versus 348 ± 5.8 ms, p = 0.09) neared significance between men and women. Transit time and PWV were not different between males and females. Baseline brachial artery diameter (0.53 ± 0.04 versus 0.43 ± 0.02 mm, p = 0.08), absolute change in diameter (0.031 ± 0.003 versus 0.027 ± 0.002 Δmm, p = 0.37), and percent dilation (5.9 ± 0.5 versus 6.5 ± 0.4%, p = 0.43) were also not significant between males and females, respectively.

Correlations were run between sodium and potassium intake and excretion and the vascular measurements for all subjects (AIx, HR-adjusted AIx, transit time, ejection duration, PWV, and FMD). Correlations were also run between sodium and potassium intake and excretion and blood pressure. There was a significant correlation between sodium intake and ejection duration ($r = -0.419$, $p < 0.05$). There was also a significant correlation between ejection duration and heart rate ($p < 0.001$, $r = -0.6$), and augmentation index and heart rate ($p < 0.01$, $r = -0.5$). There was also a significant correlation between 24-hour sodium excretion and DBP ($r = 0.462$, $p < 0.05$) as shown in Figure 5, 24-hour sodium excretion and MAP ($r = 0.40$, $p < 0.05$) as shown in Figure 6, and the excretion ratio and ED ($r = -0.45$, $p < 0.01$) as shown in Figure 7. Using a Spearman's correlation for the relationship between 24-hour sodium excretion and DBP, significance remained ($p < 0.05$). In regards to the main variable, the

sodium:potassium excretion ratio, there were no significant correlations with AIx ($r = -0.12$, $p > 0.05$; Figure 8), PWV ($r = 0.25$, $p > 0.05$; Figure 9), or FMD ($r = -0.15$, $p > 0.05$; Figure 10). There were no other significant correlations between sodium intake, potassium intake, or the intake ratio and vascular measurements (all $p > 0.05$).

When separating subjects into high (N = 25) and low (N = 5) sodium intake based upon the Dietary Guidelines for Americans (2,300 mg), there were no significant differences with the aforementioned vascular measurements (all $p > 0.05$). Because of the large difference in the number of subjects between the groups, we used the median sodium intake for the study population. When separating subjects into high and low sodium intake based upon median sodium consumption (3,049 mg), there was a significant difference with HR-adjusted AIx ($p < 0.05$) as shown in Figure 11, but no other vascular measurement. When separating subjects into high and low potassium intake based upon median potassium consumption (2,947 mg), there were no significant differences in vascular measurements. When separating subjects into high and low sodium excretion based upon median sodium excretion (134.5 mmol/d), there was a significant difference with SBP ($p < 0.05$) and DBP ($p < 0.05$) as shown in Figure 12, but no other vascular measurement. There were no significant differences when separating subjects into high and low potassium excretion based upon median potassium excretion (65.5 mmol/d) or a high and low Na+/K+ excretion ratio based upon median Na+/K+ excretion ratio (2.165) (all $p > 0.05$).

Chapter 4

DISCUSSION

The purpose of this study was to analyze the effect of habitual dietary intake of sodium and potassium on measures of vascular function in a healthy, aged population. The novelty of this study is that it utilized the 24-hour urinary sodium:potassium excretion ratio as the main variable. The hypothesis tested was that a high sodium and low potassium intake, reflected by a high sodium:potassium excretion ratio would correlate with poorer vascular function. Conversely, a low sodium and high potassium diet (as seen in the DASH diet), reflected by a low sodium:potassium excretion ratio would be associated with better vascular function. The measurement and analysis of blood pressure, PWA, PWV, brachial artery FMD, and blood and urine electrolyte levels were used.

There are several findings of this study. First, the study population consumed more sodium (3,187 ± 169 mg) and less potassium (3,120 ± 177) than recommended by the Dietary Guidelines for Americans (2,300 mg and 4,700 mg, respectively). This is important given that the average age of the subjects was 62 years old and the recommended intake of sodium is 1,500 mg per day, which was clearly exceeded. Likewise, sodium consumption correlated with sodium excretion and potassium consumption correlated with potassium excretion

illustrating that subjects kept accurate dietary records. Sodium excretion correlated with a higher mean arterial pressure and diastolic blood pressure although none were hypertensive. Finally, when subjects were separated into a high and low sodium excretion group, there was a statistical difference between systolic and diastolic pressure and sodium excretion.

4.1 Dietary Intake of Sodium and Potassium

Current dietary recommendations for sodium intake are 2,300 mg for the general population and 1,500 mg for special populations such as people over 51 years old, hypertensives, and African Americans (CDC, 2009). The NHANES data from 2005-2006 estimates that the average intake for males over the age of 50 years was 3,900 mg, and the estimated average intake for females over the age of 50 years was 3,000 mg (NHANES, 2008). In the current study, on average, males consumed 3,471 ± 261 mg, which is lower than the current average but still greatly exceeds recommendations (231% of special population recommendation, 151% of general population recommendation). The older females consumed 2,861 ± 169 mg, which is also lower than the current average but much higher than the recommended (191% of special population recommendation, 124% of general population recommendation).

The dietary recommendations for potassium intake are 4,700 mg (CDC, 2009). Actual potassium intake is much lower than recommended values. Currently in the U.S., men consume an average of 3,159 mg/d of potassium or

68% of recommended intake, while women consume an average of 2,379 mg/d or 50% of recommended intake (What We Eat in America; Potassium Intake, 2010). In the current study, on average men consumed 3,307 ± 256 mg (70% of recommended intake), and women consumed 2,907 ± 229 mg (62% of recommended population) suggesting that our subjects ate more potassium rich foods than the average U.S. adult and in particular, this was the case when it came to the women. Therefore, the potassium intakes of this study are consistent with current consumption values for the men and greater for the women as seen in the NHANES and CDC data (NHANES, 2008; CDC, 2009).

When subjects were divided into a high and low sodium intake group based upon median sodium intake of the data's subject population (3,049 mg), heart rate-adjusted AIx was significantly lower in the high sodium intake group, while SBP, DBP, ejection duration, and AIx were approaching significance. Although there was a significant difference in HR-adjusted AIx between groups, sex (and thereby height) may have influenced the outcome. There were far less females (N = 5) in the high salt group than males (N = 10), while the low salt group had more females (N = 9) than males (N = 6). Females are generally shorter in stature, and thereby the reflected wave returns sooner, causing an increase in AIx. Yasmin and Brown (1999) found that there was a several fold higher AIx in women than men and the difference was explained in part by an inverse regression correlation between AIx and height. They mentioned that more extensive research should be performed to determine other influential

variables to describe the difference between males and females in regards to AIx. This is likely the reason why HR-adjusted AIx is greater in the low sodium intake group.

Along with vascular measurements, many studies have found a correlation between sodium intake and blood pressure (Khaw and Barrett-Connor, 1988, Sacks et al., 2001; INTERSALT, 1988; Cook et al., 2007 and 2009;; Trials of Hypertension, 1997). Khaw and Barrett-Connor (1998) found that in normotensive and hypertensive subjects aged 30-79, age-adjusted SBP and DBP correlated significantly with the sodium:potassium intake ratio in both men and women. In addition, there was a graded change in the regression slope for BP versus sodium:potassium intake ratio as it increased with increasing age, suggesting increasing sensitivity to the sodium:potassium intake ratio with age. In the current study, sodium intake did not correlate with blood pressure however sodium excretion did.

4.2 Sodium and Potassium Excretion

In the current study, the average sodium excretion over 24 hours was 145.6 ± 10.5 mmol/d, with the range of sodium excretion being 73.8 – 299.3 mmol/d. When examining the data between the males and females in the current study, a few significant differences were found. Urinary sodium excretion over 24 hours was significantly higher in males than females, as urinary potassium excretion over 24 hours was approaching the same significance ($p = 0.06$);

however, the excretion ratio did not reach significance. This indicates that on average, males were eating more sodium and potassium, likely due to a higher caloric intake. Although there was no significant difference between males and females with total energy intake, on average males had a higher energy intake than females (males – 2,350 $\pm$ 220 kcal, females – 1,952 $\pm$ 147 kcal).

O'Donnell et al (2011) examined cardiovascular risk as it related to sodium intake, measured by sodium excretion. Using the ONTARGET and TRANSCEND trials, they estimated the 24-hour sodium excretion from a morning fasting urine sample, and confirmed previous literature in that sodium excretion and cardiovascular events are depicted in a J-shaped curve. Unfortunately, the current data did not have a large enough sodium intake range to be able to plot a j-shaped curve. In addition, researchers also delineated quintiles for sodium excretion as it relates to cardiovascular risk and mortality. Of the current data, 4 subjects fell into the lowest quintile (< 87 mmol/d), 21 subjects fell into the second quintile (87 – 174 mmol/d), 2 subjects fell into the third quintile (175 – 260 mmol/d), 3 subjects fell into the fourth quintile (260 – 348 mmol/d), and no subjects fell into the highest quintile (> 348 mmol/d). In total, more than 80% (N = 25) of the current study's subjects were excreting optimal sodium, whereby they are at very-low or low risk of mortality. In regards to seeing dramatic differences in vascular function or hemodynamics, these data show a low average and small range of sodium excretion, and therefore differences in vascular function may have been unlikely to occur given the narrow range of

sodium excretion. This factor is certainly a limitation of the current study. Although the current study was cross-sectional and therefore, not able to evaluate mortality and its relationship with sodium excretion (as the O'Donnell study did), there was a significant correlation between sodium excretion and both DBP and MAP.

Likewise, when separating the subjects into high and low sodium excretion groups based upon median sodium excretion of the subject population (135 mmol/d), there were significant differences between groups with regards to SBP and DBP. Intersalt (1996) found similar outcomes when analyzing 52 population samples aged 20-59. They found that 24-hour urinary excretion higher by 100 mmol was associated with a blood pressure higher on average by 5-7/2-4 mmHg, and this relationship was stronger with an older population (40-59 years). The current data's difference between high and low average sodium excretions was 83 mmol/d (high – 187 mmol/d, low – 104 mmol/d), and the concurrent blood pressure average difference was 8/7 mmHg. In total, although more than 80% of the current subjects fell into the lowest or low-risk cardiovascular mortality categories, we were able to find a difference in vascular function when separating out low and high sodium excretion. So, even if cardiovascular mortality may not be an issue when comparing to sodium excretion in an older population, vascular function is compromised. A wider range of sodium excretions is needed to further look at these comparisons.

The average potassium excretion for this study was 73.6 ± 5.5 mmol/d, with the range being 24.3 to 140.6 mmol/d. Again, O'Donnell et al (2011) delineated quintiles for potassium excretion as it relates to cardiovascular risk and mortality. Of the current data, 3 subjects fell into the lowest quintile (< 38.4 mmol/d), 5 subjects fell into the second quintile (38.5 – 50.9 mmol/d), 5 subjects fell into the third quintile (51.0 – 63.7 mmol/d), 5 subjects fell into the fourth quintile (63.8 – 76.7 mmol/d), and 12 subjects fell into the highest quintile (> 76.8 mmol/d). These data suggest that there was a more even spread of potassium excretion than sodium excretion among our older adults. However, research is not consistent as to the prediction of cardiovascular events or mortality and potassium excretion; rather, most research emphasizes analyzing sodium and potassium together, instead of each, individually.

In regards to the sodium:potassium excretion ratio, the average for the current study was 2.2 ± 0.14, with a range of 0.801 to 3.933. Of these values, 20 subjects fell within 1 SD of the mean, and 10 subjects fell within 2 SD from the mean. As with potassium excretion, the excretion ratios were very condensed and values were low, likely contributing to lack of relationship with the vascular measurements. Much like the quintiles from the work done by O'Donnell et al., Huggins et al (2011) evaluated the sodium:potassium excretion ratio in a cross-sectional study in an Australian population using the Melbourne Collaborative Cohort Study (average age of 64); however, they used HTN as the main outcome measure instead of mortality. They determined that a higher excretion ratio was

associated with a higher blood pressure. The current study did not find such significance. Of the current data, 5 subjects fell into the lowest quintile (< 1.31), 5 subjects fell into the second quintile (1.31 – 1.67), 3 subjects fell into the third quintile (1.68 – 2.06), 9 subjects fell into the fourth quintile (2.06 – 2.61), and 8 subjects fell into the highest quintile (> 2.61). Although the current study did not analyze the sodium:potassium excretion ratio and mortality, subjects were generally spread evenly among the mortality risk quintiles.

When dividing subjects into high and low potassium excretion based upon median potassium excretion of the subject population (65 mmol/d), there were no significant differences between groups. Many studies have shown a marked increase in blood pressure with low dietary intake or a decrease in potassium from the recommended intakes (van Mierlo et al., 2010; Dolson et al., 1995; Geleijnse et al., 2003; INTERSALT, 1988). The INTERSALT (1988) study found that a decrease in potassium excretion by 50 mmol/d increased SBP 3.4 mmHg and increased DBP 1.9 mmHg. Although the current study did not find any significant differences between groups, it may be due to the small difference between average high (83 mmol/d) and low (62 mmol/d) potassium excretion between groups. The INTERSALT (1988) study examined a significant change in blood pressure with a larger potassium excretion difference (50 mmol/d) than the current study (21 mmol/d). A larger excretion spread may be necessary to see significant differences.

4.3 Augmentation Index

In regards to augmentation index, Park et al (2011) found that sodium excretion and the sodium:potassium excretion ratio was independently associated with AIx in 515 hypertensive individuals. Although the current data did not find significance between sodium excretion or the sodium:potassium excretion ratio and AIx, the current study's' subjects were normotensive, which may have dampened the effects seen, as there is a higher capacity for improvement with hypertensive individuals. Also, females had a significantly higher heart rate-adjusted AIx than the male subjects. On average, AIx and ejection duration were trending towards being significantly greater in females as well. However, higher AIx in females is expected and supported in the literature, as AIx inversely correlates with height, and females on average, are shorter (Yasmin and Brown, 1999; London et al., 1992). In the study by Yasmin and Brown (1999), after performing a multiple regression analysis, it was shown that increased AIx in women is only partly explained by a shorter height. They concluded that future research should focus on other variables that contribute to the difference. Some studies have found a greater correlation between AIx and PWV within genders rather than both genders combined, suggesting that gender is a confounding variable (Yasmin and Brown, 1999; Marchais et al., 1993). Albeit, in this study there were no significant correlations between either AIx or HR-adjusted AIx and PWV within genders or both genders combined, on average

women had higher AIX, HR-adjusted AIx, and PWV values. Again, the small sample size in the current study may have been a limiting factor.

As in our study, research has shown that vascular function decreases not only in a diseased population, but also in a healthy, older population. Nurnberger et al (2002) found that in a healthy population free of atherosclerosis, AIx significantly correlates with age. In the current study, ejection duration (ED), a product of PWA significantly correlated with the sodium:potassium excretion ratio and sodium intake, and was approaching significance with sodium excretion. In the literature, Wilkinson et al (2000) found that ED is influenced by heart rate. In the current data, there was a significant correlation when examining the relationship between sodium excretion and ED ($r = -0.549$, $p < 0.01$) and the sodium:potassium excretion ratio and ED ($r = -0.373$, $p < 0.05$), while controlling for HR. This indicates that increased sodium excretion and excretion ratio are related to increased ED. It is unclear what physiological relevance this relationship has and whether the dietary intake of sodium and potassium influences ED at all. Also, when separating subjects into high and low sodium intake groups based upon median sodium intake, there was a significant relationship between HR-adjusted AIx and level of sodium intake, as well as a trending relationship between AIx and sodium intake ($p = 0.06$). To our knowledge, no other study has reported this relationship.

The current study did not find a significant correlation between Aix or HR-adjusted Aix and potassium intake or excretion for all subjects together. This is

consistent with some literature. Matthesen et al (2012) used a wide age-range, healthy group and supplemented them with 3,900 mg of potassium per day, for 28 days. They found that neither AIx nor BP was significantly changed by potassium supplementation.

4.4 Pulse Wave Velocity

When dividing subjects into high and low sodium:potassium excretion ratio groups based upon the median ratio of the subject population (2.165), PWV was approaching significance between the two groups ($p = 0.08$). No other relationship among vascular measurements was found. Although the current study did not find any significant correlations between sodium or potassium intake or excretion and PWV, some literature has found such a relationship. The current study only found a trending significant difference in PWV when separating the subjects into high and low excretion ratios groups based upon the median excretion ratio. In a study by Polonia et al (2006), researchers assessed 24-urine collections and vascular function in 426 (50 ± 22 years old), hypertensive and normotensive Portuguese individuals. They found that sodium intake has a significant effect on aortic stiffness, by assessment of PWV. Contrary to most research, Garcia-Ortiz et al (2012) found that sodium intake negatively correlated with PWV. It should be noted that over half of the subjects were hypertensive and one-third were diabetic. In certain disease populations, it has been suggested that a low sodium diet may be detrimental and therefore, a

J-shaped curve exists between sodium intake and cardiovascular outcomes (Alderman & Cohen, 2012). While the current study did not find significance with sodium intake and PWV, the relationship was positive, suggesting that a higher sodium intake is associated with a faster pulse wave. Few studies have looked at potassium intake and PWV. Matthesen et al (2012) used a wide age-range, healthy group and supplemented them with 3,900 mg of potassium per day, for 28 days, and found that PWV increased slightly, but not significantly. The current study looked at habitual intake and therefore, it is unknown whether PWV would have changed in our subjects if we had increased their potassium intake.

4.5 Brachial Artery Flow-Mediated Dilation

The current data showed that, on average, women had a higher AIx and PWV, but conversely a lower FMD. Brachial artery FMD did not correlate with any measures in the current study, however, correlations with FMD have been seen throughout the literature. Previous research has shown that sodium intake affects FMD; however, there were only few studies that strictly used a healthy, older population. Jablonski et al., (2009) found that in a 48-73 year old population, FMD was enhanced in a low sodium consumption group (<2,300 mg/d). However, this study used both normotensive subjects and stage 1 hypertensive subjects while the current study used only normotensive individuals. Likewise, another recent study of healthy individuals examined the postprandial effects of a high salt diet versus a low salt diet on FMD and found that FMD was

impaired just 30 minutes after consumption of a high salt meal in an 18-70 year old group (Dickinson et al., 2011). This finding is similar to a diet controlled study that found that FMD was higher following 2 weeks of a high salt diet as opposed to a low salt diet (Dickinson et al., 2009). To date, no studies have evaluated the direct effect of potassium intake on FMD; however, FMD has been shown to improve in older adults with moderately elevated SBP when following the DASH diet (Blumenthal et al., 2010).

4.6 Perspectives

It is well known that across age groups, and in both seemingly healthy and diseased populations, reduced sodium intake and increased potassium intake, indicated by a decreased sodium:potassium excretion ratio has beneficial effects on BP and vascular function. Unpublished work by Schellhardt et al., (2012) found that in healthy, young males a low sodium:potassium excretion ratio was associated with a better Aix however no relationship was found between the excretion ratio and PWV or FMD, though. The current study found similar relationships using an older population. This indicates that sodium and potassium excretion within the range observed in the current study did not correlate with vascular function in this older, healthy population. The relationship of sodium and potassium intake with vascular function has been shown to fall into a J-shaped curve for diseased populations, whereby too little or too much consumption can degrade vascular function (Garcia-Ortiz et al., 2012). The

current study was novel in that it looked at habitual dietary intake of sodium and potassium in a healthy, older population (average age – 62), and its effects on vascular function. The lack of significance between intake and excretion with vascular function may be due to the small cohort used, the condensed ranges of sodium and potassium intake, or the predisposed good health of the subject population. Likewise, the majority of studies in the area are intervention studies that manipulate sodium or potassium intake, while our study looked at the habitual dietary intake of individuals. This study did, however find significance with blood pressure and sodium excretion. This finding is prevalent in the literature. Interestingly, in a population with CVD (2,096 of the 3,681 were normotensive), Stolarz-Skrzypek (2011) determined that SBP, but not DBP, changes over time aligned with changes in sodium excretion, but this association did not translate into a higher risk of HTN or CVD complications. Most studies have employed diseased populations, as they have more capacity to improve their vascular function; however it would be interesting to see differences with vascular function and sodium or potassium intake or excretion in a healthy, older population.

There were several limitations of this study. First, the study population for a cross-sectional study was relatively small. Also, this study used a self-recorded habitual dietary intake using a three day food record. Subjects were instructed on approximating portion sizes, however, there is inherent error in self-reported dietary intake. Nevertheless, our sodium and potassium excretion data

does correlate with our intake data. Likewise, subjects may not have collected every urine deposit during the 24-hour collection process. Although instructed to record the time of the first and last urine deposit, some subjects did not abide. Therefore, subjects with no recorded time were allotted 24 hours by default. There was also a very condensed range of sodium and potassium intakes and excretions, which could have contributed to the lack of differences with vascular measurements. Lastly, this study did not look at any potential mechanisms as to the differences in vascular function or hemodynamic values with regards to different levels of sodium or potassium intake or excretion.

4.7 Conclusions

The findings of the current study are unable to support the idea that the sodium:potassium excretion ratio, potassium intake, or sodium or potassium excretion influence vascular function by measurement of AIx, PWV, or brachial artery FMD. However, blood pressure seems to be influenced by sodium excretion, and heart rate-adjusted augmentation index seems to be influenced by sodium intake. Further research needs to be performed to delineate significant relationships with regards to vascular measurements (PWA, PWV, and FMD).

TABLES

Table 1: Subject Characteristics

	Men	Women	All subjects
Number	16	14	30
Age (years)	62 ± 2	62 ± 2	62 ± 1
Height (cm)	179.9 ± 1.8	165.9 ± 1.7 *	173.4 ± 1.8
Body Mass (kg)	89.1 ± 4.1	71.7 ± 3.2 *	89.0 ± 3.1
BMI (kg/m2)	27.3 ± 0.9	25.9 ± 0.9	26.6 ± 0.7
Systolic BP (mmHg)	122 ± 3	118 ± 2	120 ± 2
Diastolic BP (mmHg)	74 ± 2	69 ± 2	72 ± 2
Heart Rate (bpm)	60 ± 2	60 ± 2	60 ± 2
Hemoglobin (g/dL)	15.3 ± 0.27	13.7 ± 0.35 *	14.4 ± 0.25
Hematocrit (%)	44 ± 1	40 ± 1 *	42 ± 1
Active Energy Expenditure (kcal)	300 ± 29	325 ± 33	313 ± 22

All data reported as mean ± SEM. * $p<0.05$ vs. men

Table 2: Blood and Urine Values

	Men	Women	All Subjects
Urine Volume (ml)	1,567 ± 167	1,856 ± 236 *	1,720 ± 149
Serum Sodium (mmol/L)	140 ± 0.9	140 ± 0.7	140 ± 0.6
Serum Potassium (mmol/L)	4.3 ± 0.07	4.2 ± 0.07	4.2 ± 0.05
Plasma Osmolality (mOsm/L)	293 ± 1.9	291 ± 1.4	293 ± 1.2
Urinary Sodium Excretion/24 hrs (mmol/d)	176.8 ± 14.9	110.0 ± 7.1 *	145.6 ± 10.5
Urinary Potassium Excretion/24 hrs (mmol/d)	83.4 ± 7.6	62.4 ± 6.8	73.6 ± 5.5
Sodium: Potassium Excretion Ratio	2.3 ± 0.17	1.9 ± 0.19	2.2 ± 0.14

All data reported as mean ± SEM. * $p<0.05$ vs. men

Table 3: Habitual Dietary Intake

	Men	Women	All subjects
Total Energy (kcal)	2350 ± 220	1940 ± 147	2159 ± 141
Total CHO (g) **% of total kcal**	273.7 ± 25.4 45.5 ± 2	219.8 ± 15.1 44.9 ± 2	248.5 ± 16.0 45 ± 1
Total PRO (g) **% of total kcal**	93.1 ± 8.3 17.7 ± 2	79.6 ± 6.3 16.5 ± 1	86.8 ± 5.5 17 ± 1
Total Fat (g) **% of total kcal**	94.3 ± 11.3 33.6 ± 2	82.0 ± 9.5 36.2 ± 2	88.5 ± 7.6 35 ± 2
Sodium (mg)	3471 ± 261	2861 ± 169	3187 ± 169
Potassium (mg)	3307 ± 256	2907 ± 229	3120 ± 177
Sodium:Potassium Intake Ratio	1.1 ± 0.09	1.1 ± 0.09	1.1 ± 0.06
Calcium (mg)	1150 ± 158	984 ± 140	1073 ± 108
Magnesium (mg)	374 ± 34	324 ± 29	350 ± 23
Phosphorous (mg)	1413 ± 157	1297 ± 125	1359 ± 103
Caffeine (mg)	212 ± 35	186 ± 43	199 ± 27
Alcohol (g)	12.4 ± 4.9	6.5 ± 2.6	9.7 ± 2.9

All data reported as mean ± SEM. CHO (carbohydrates); PRO (protein).

Table 4: Vitamin Intake

	Men	Women	All Subjects
Vitamin A (mcg)	1506 ± 205	1419 ± 278	1417 ± 170
Vitamin D (mcg)	7.01 ± 1.47	5.75 ± 0.94	6.56 ± 0.90
Vitamin E (mg)	12.91 ± 1.75	9.27 ± 1.56	11.35 ± 1.23
Vitamin K (mcg)	177 ± 51	205 ± 67	188 ± 42
Thiamin (mg)	2.12 ± 0.16	1.48 ± 0.09 *	1.83 ± 0.11
Riboflavin (mg)	2.80 ± 0.28	2.24 ± 0.21	2.55 ± 0.18
Niacin (mg)	29.5 ± 2.7	21.5 ± 1.4 *	26.2 ± 1.8
Folate (mcg)	534 ± 52	425 ± 34	483 ± 34
Vitamin B6 (mg)	2.72 ± 0.29	1.80 ± 0.13 *	2.30 ± 0.19
Vitamin B12 (mcg)	5.71 ± 1.02	4.48 ± 0.46	5.26 ± 0.59
Vitamin C (mg)	130 ± 15	118 ± 20	123 ± 13

All data reported as mean ± SEM. * $p < 0.05$ vs. men

Table 5: Vascular Measurements

	Men	Women	All subjects
Augmentation Index (%)	26 ± 2.8	33 ± 2.4	29 ± 2.0
Augmentation Index HR Adjusted (%)	19 ± 2.5	26 ± 2.1 *	22 ± 1.8
Ejection Duration (ms)	335 ± 4.6	348 ± 5.8	341 ± 3.8
Transit Time (ms)	149 ± 2.8	142 ± 4.8	146 ± 2.8
PWV (m/s)	7.6 ± 0.44	7.8 ± 0.48	7.7 ± 0.32
Baseline Brachial Artery Diameter (mm)	0.53 ± 0.04	0.43 ± 0.02	0.48 ± 0.02
Brachial Artery FMD (absolute Δ mm)	0.031 ± 0.003	0.027 ± 0.002	0.028 ± 0.02
Brachial Artery FMD (% dilation)	5.9 ± 0.5	6.5 ± 0.4	6.2 ± 0.3

All data reported as mean ± SEM. Pulse Wave Velocity (PWV); Flow-Mediated Dilation (FMD); *p<0.05 vs. men

FIGURES

Figure 1: Timeline of Data Collection

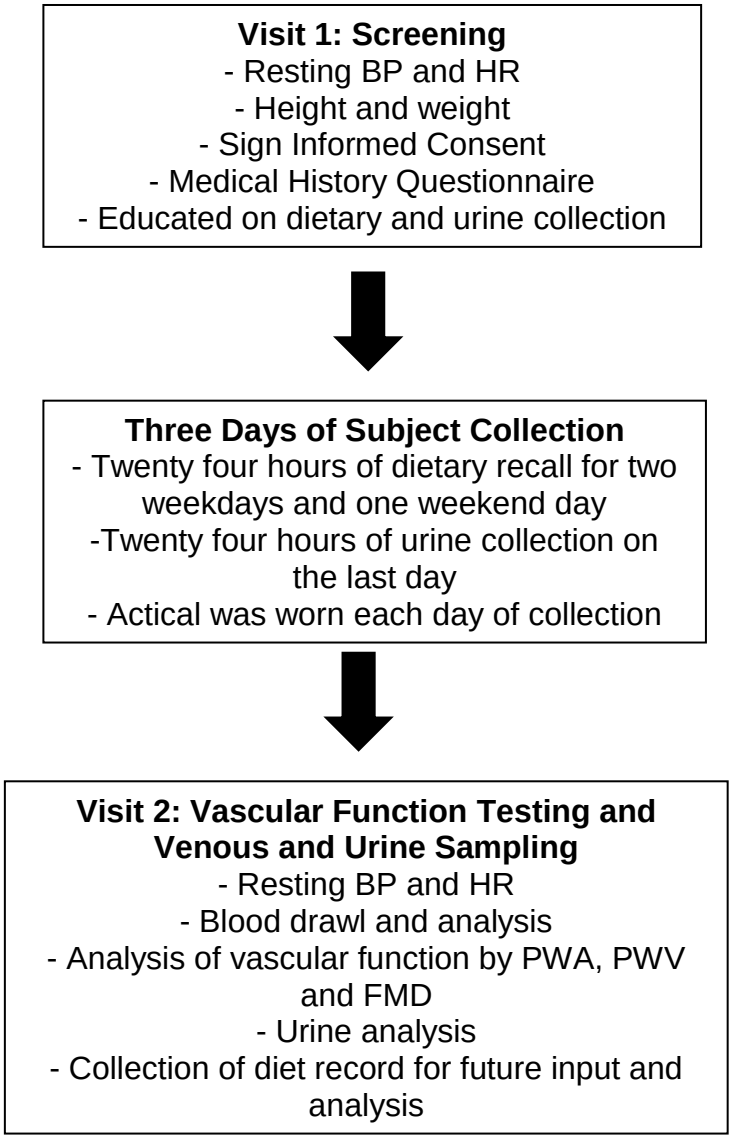


Figure 2: Sodium Intake versus Potassium Intake in Males and Females

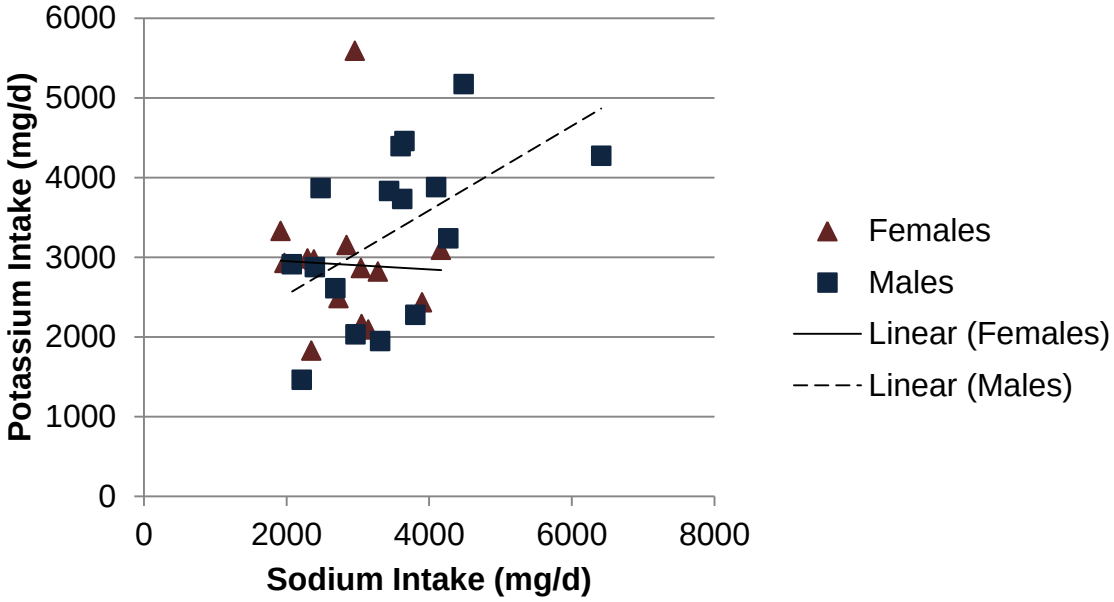


Figure 3: Association between Sodium Intake (mg/d) and Sodium Excretion (mmol/d)

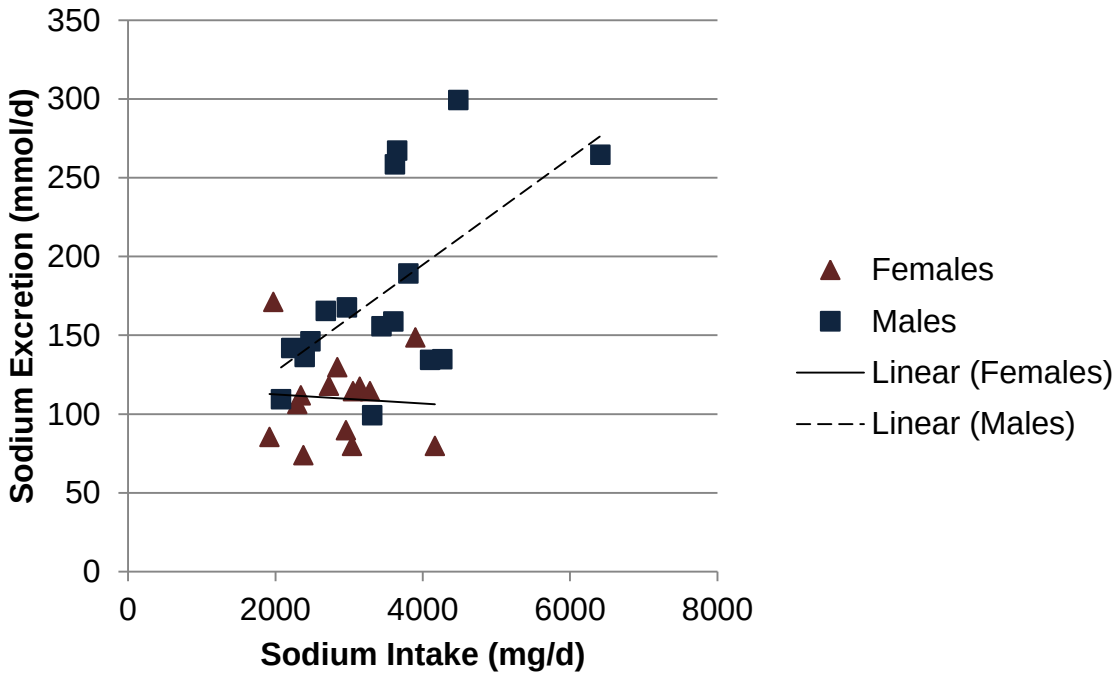


Figure 4: Association between Potassium Intake (mg/d) and Potassium Excretion (mmol/d)

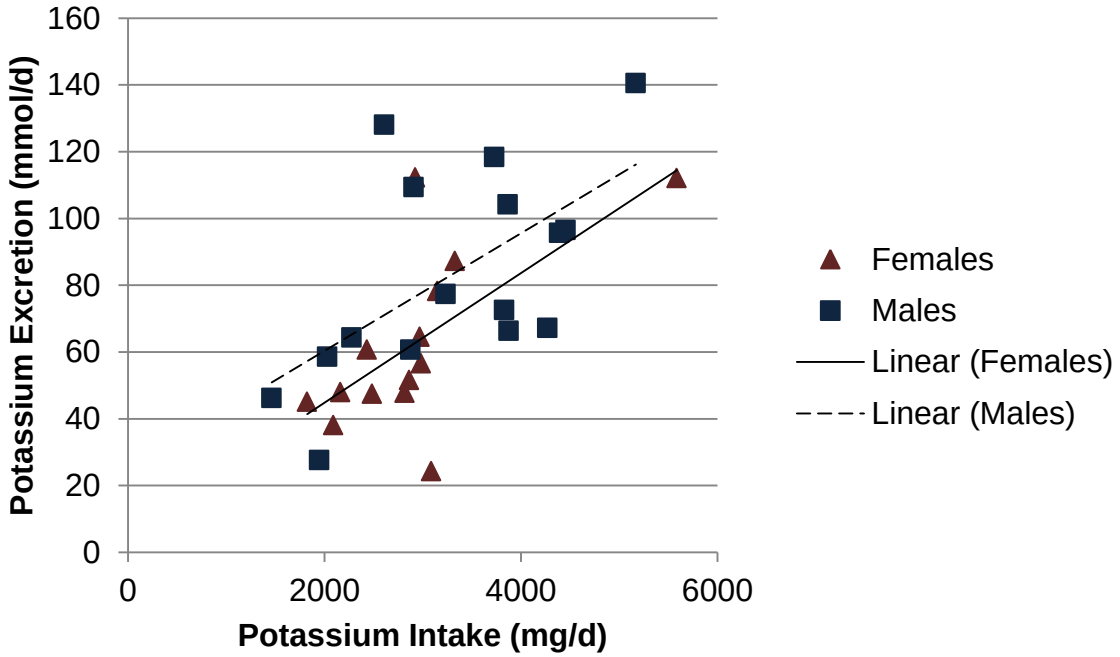


Figure 5: 24-hour Sodium Excretion versus Diastolic Blood Pressure in All Subjects

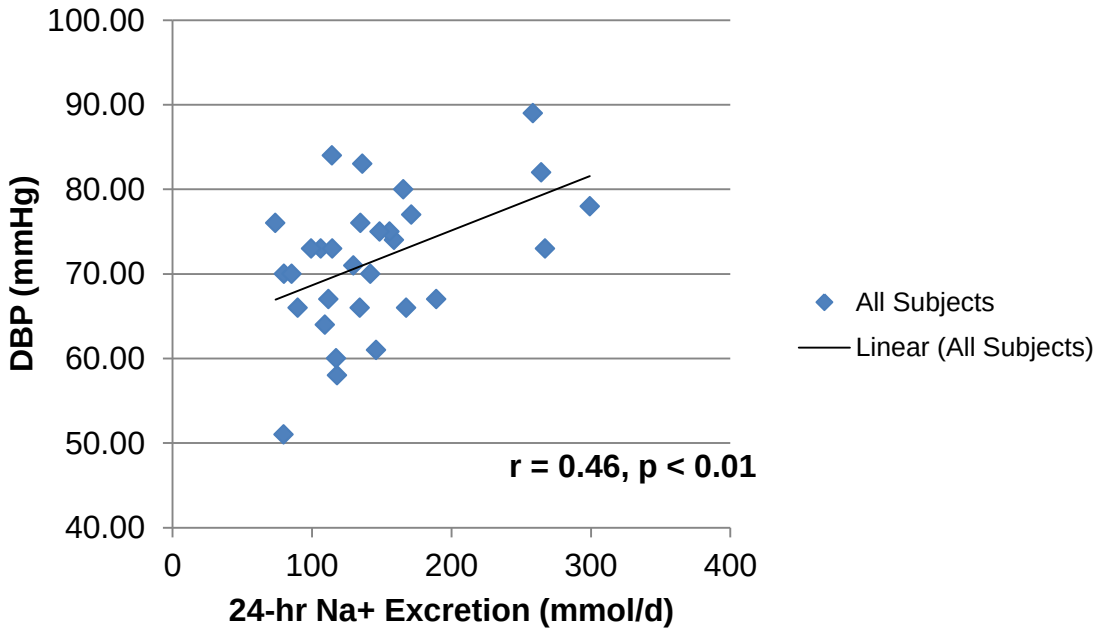


Figure 6: 24-hour Sodium Excretion versus Mean Arterial Pressure (MAP) in All Subjects

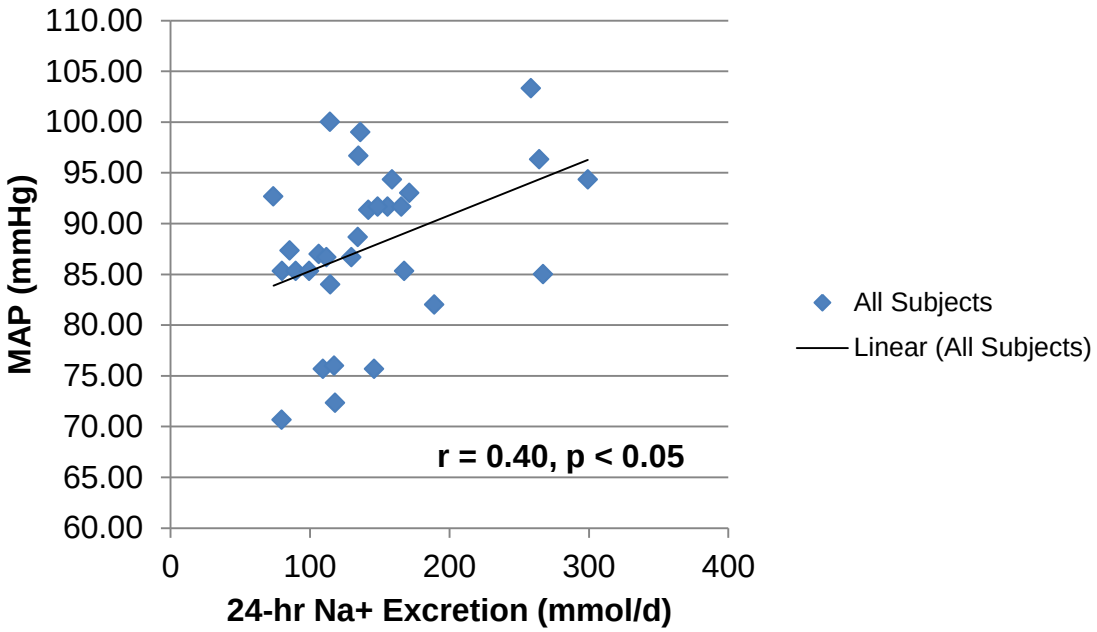


Figure 7: Sodium:Potassium Urinary Excretion Ratio versus Ejection Duration in all Subjects

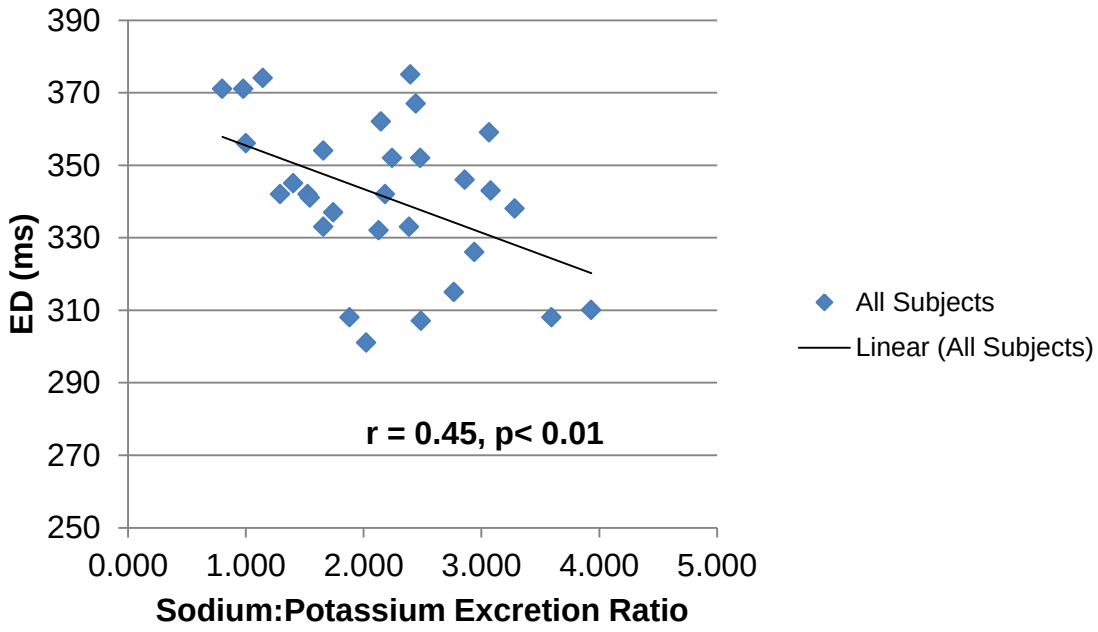


Figure 8: Sodium:Potassium Urinary Excretion Ratio versus Augmentation Index in all Subjects

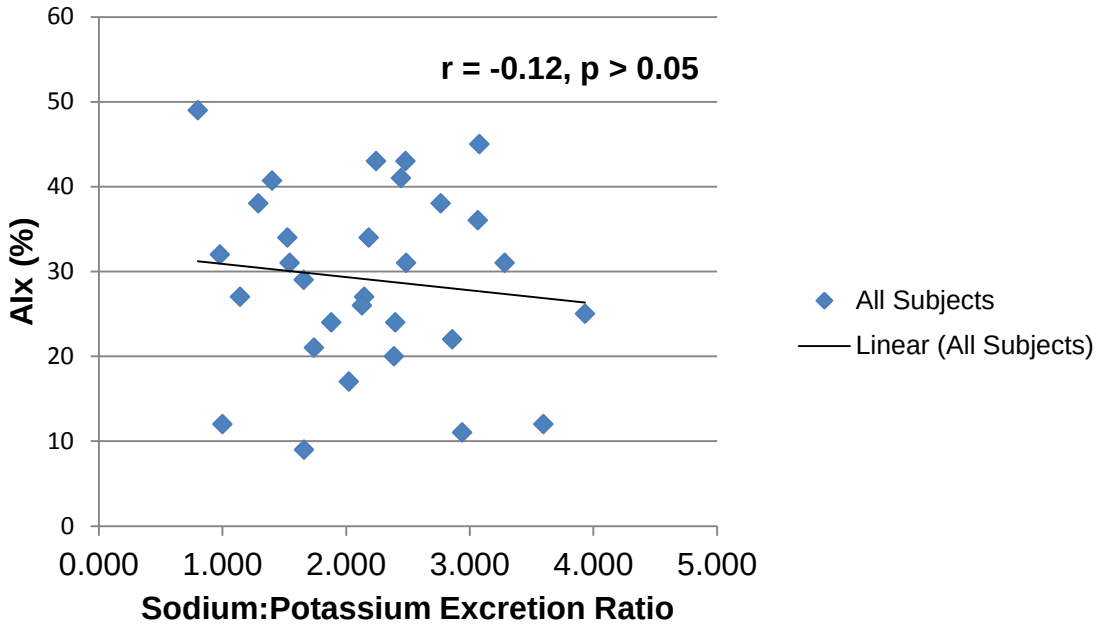


Figure 9: Sodium:Potassium Urinary Excretion Ratio versus Pulse Wave Velocity in All Subjects

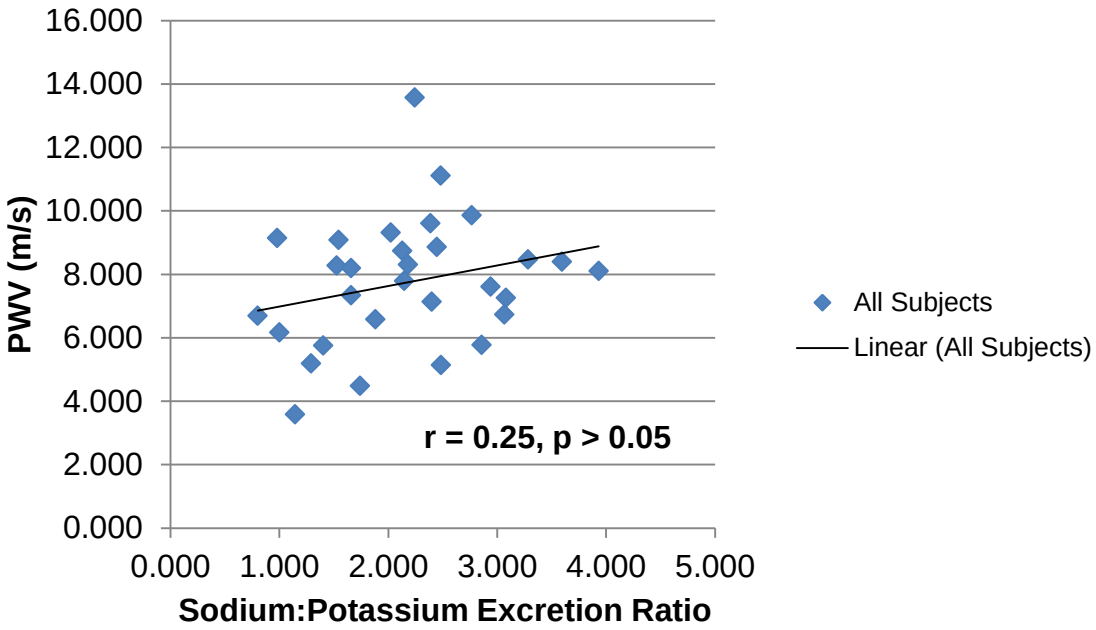


Figure 10: Sodium:Potassium Urinary Excretion Ratio versus Brachial Artery Flow-Mediated Dilation in All Subjects

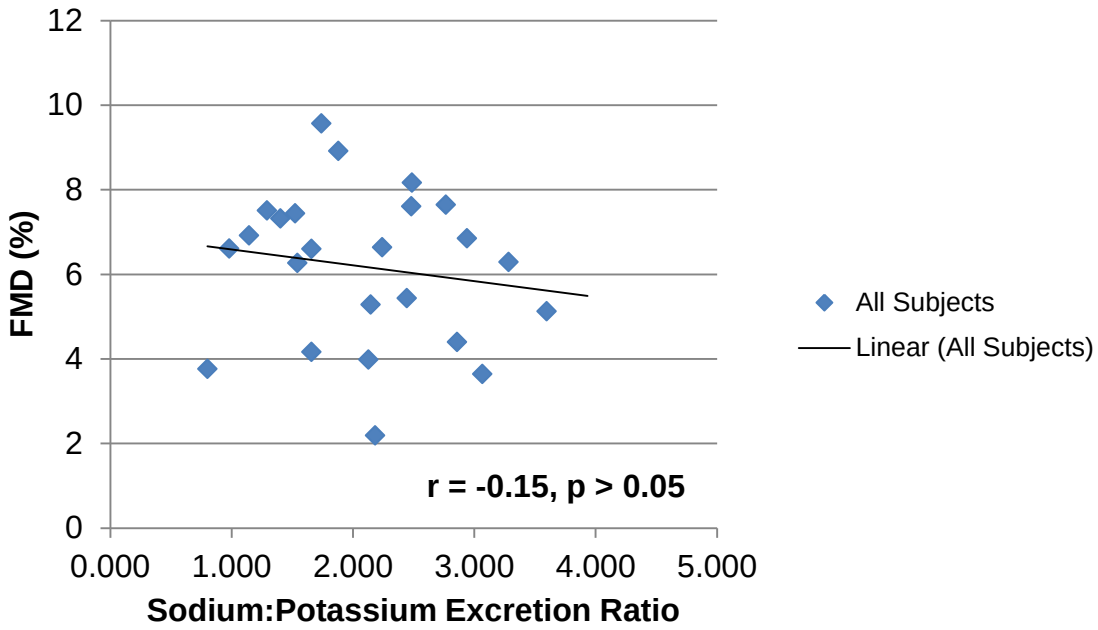


Figure 11: Heart Rate-Adjusted Augmentation Index between High and Low Sodium Intake

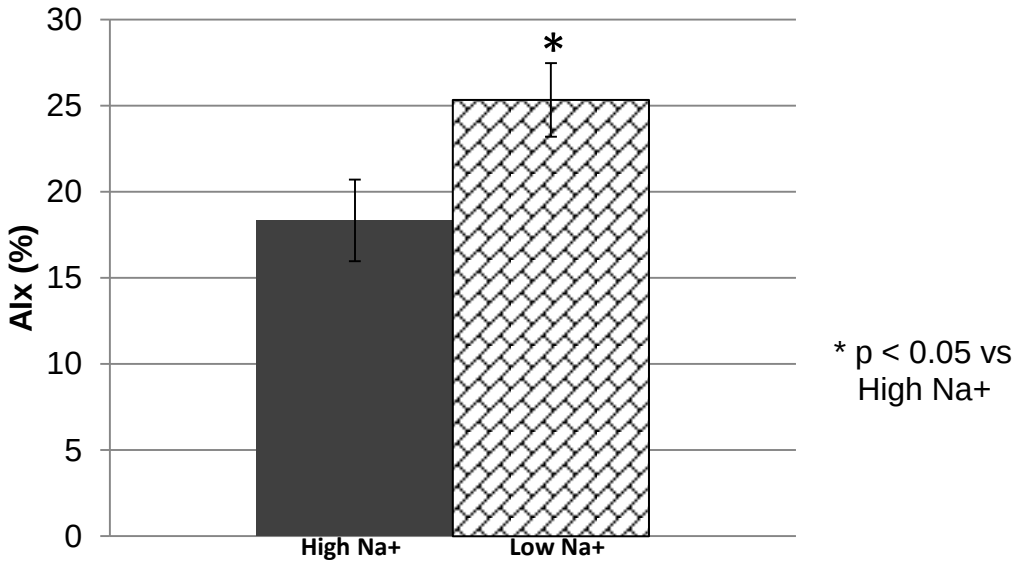
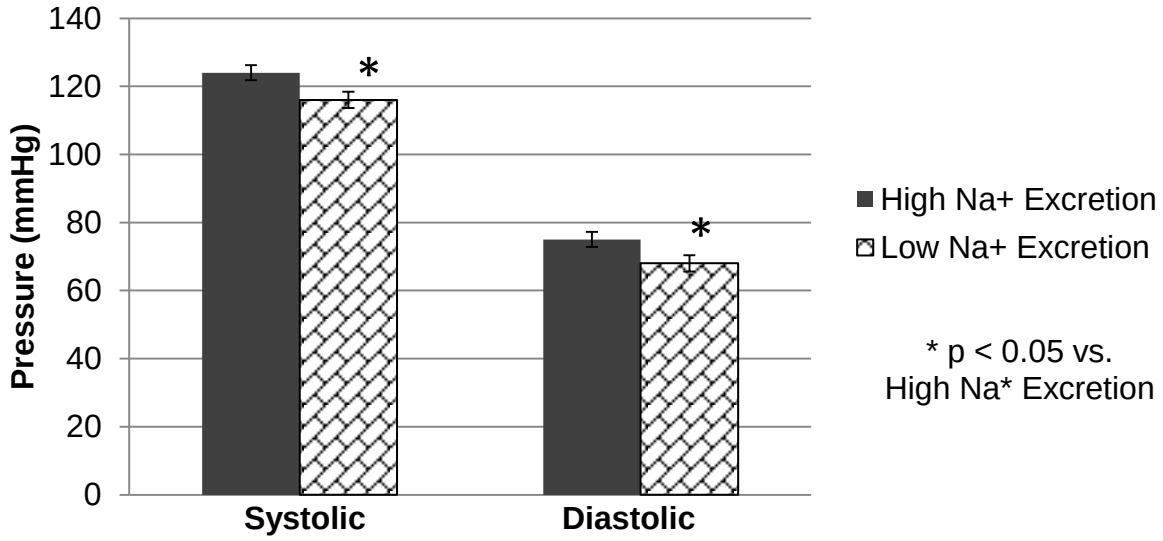


Figure 12: Blood Pressure between High and Low Sodium Excretion



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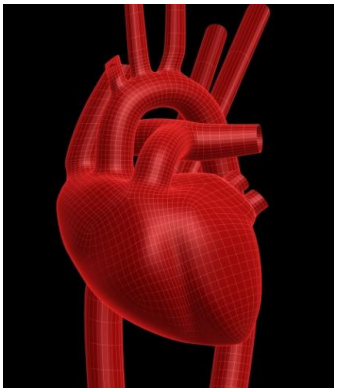
Appendix A

RECRUITING ADVERTISEMENT

We are looking for volunteer participants for a research study examining the relationship between diet and blood vessel function



You are eligible if you are:

- Between the ages of 50-80
- Are healthy, and have a normal blood pressure

For more information, please contact Brittany at (484) 802-6852
or ballman@udel.edu

We look forward to hearing from you!

Conducted jointly by the Department of Behavioral Health and Nutrition and the Department of Kinesiology and Applied Physiology

Brittany at (484) 802-6852 ballman@udel.edu	**Brittany at (484) 802-6852 ballman@udel.edu**	**Brittany at (484) 802-6852 ballman@udel.edu**	**Brittany at (484) 802-6852 ballman@udel.edu**	**Brittany at (484) 802-6852 ballman@udel.edu**	**Brittany at (484) 802-6852 ballman@udel.edu**	**Brittany at (484) 802-6852 ballman@udel.edu**	**Brittany at (484) 802-6852 ballman@udel.edu**	**Brittany at (484) 802-6852 ballman@udel.edu**	**Brittany at (484) 802-6852 ballman@udel.edu**

Appendix B

MEDICAL HISTORY QUESTIONAIRRE

25 North College Ave
McDowell Hall, Room 119
Phone: 302-831-3195 Fax: 302-831-3193
www.udel.edu/nmhc

Research Participant Medical Questionnaire

I. Personal Information

First Name: _____ Middle Name: _____

Last Name: _____

Home Street Address: _____

City, State, Zip: _____

Last 4-digits of SSN: _____ Date of Birth: _____

Gender: ❑ Male ❑ Female Age: _____

Marital Status: ❑ Married ❑ Single ❑ Divorced ❑ Widowed ❑ Legally Separated ❑ Other

Race: ❑ Caucasian ❑ Black ❑ Hispanic ❑ Asian ❑ Native American ❑ Pacific Islander

❑ Asian Pacific American ❑ Alaskan Native ❑ Black-Non Hispanic

❑ White-Non Hispanic ❑ Other (supply name) _____

Personal ph#: _____ Work ph# _____

Email Address: _____

Emergency Contact

First Name: _____ Personal ph#: _____

Last Name: _____ Work ph#: _____

*How are **you** related to the Emergency Contact?*

You are the: ❑ Spouse ❑ Parent ❑ Son/Daughter ❑ Grandchild ❑ Niece/Nephew

❑ Aunt/Uncle ❑ Employee ❑ Other _____

II. Personal Physician Contact Information

Do you have a personal physician: ❑ Yes ❑ No

Physician's Name _____

Physician's Address: _____

City: _____ State: _____ Zip: ___

Do you have a nephrologist (kidney doctor) or cardiologist (heart doctor)? ❑ Yes ❑ No

Physician's Name _____

Physician's Address: _____

City: _____ State: _____ Zip: ___

III. Personal and Medical Information			
In what research study are you participating?	❑ Vitamin B6 ❑ EPR ❑ Dietary Salt ❑ Skin Blood Flow ❑ FastFES ❑ FAM ❑ Sodium/Potassium Uptake		
Check any of the medical conditions listed that **you** have been diagnosed with:	❑ High Blood Pressure ❑ Heart Disease ❑ High Cholesterol ❑ Kidney Disease	❑ Asthma ❑ Emphysema ❑ Cancer ❑ Anemia	❑ Diabetes ❑ Stroke ❑ Blood Clots
List any other medical diagnosis you have:			
Have you been hospitalized for any significant injury or illness: ❑ Yes ❑ No If **yes** list reason and dates:			

Check any of the medical conditions listed that either your **F**ather or **M**other have been diagnosed with:	**F M**	**F M**	**F M**
	❑ ❑ High Blood Pressure ❑ ❑ Heart Disease ❑ ❑ High Cholesterol ❑ ❑ Kidney Disease	❑ ❑ Asthma ❑ ❑ Emphysema ❑ ❑ Cancer ❑ ❑ Anemia	❑ ❑ Diabetes ❑ ❑ Stroke ❑ ❑ Blood Clots

If you are currently taking any prescription medicine, over-the-counter medicine, vitamins, herbs, nutritional supplements or birth control pills, please list the medication **name, dosage and frequency** taken below

1.	5.
2.	6.
3.	7.
4.	8.

Are you **ALLERGIC** to any medication, food or latex?	❑ Yes ❑ No	If yes what?	Type of reaction:
Do you smoke?	❑ Yes ❑ No	If yes how much?	How many years?
Did you ever smoke?	❑ Yes ❑ No	If yes, quit date?	# Yrs. smoked?
Do you drink alcohol?	❑ Yes ❑ No	If yes how much?	How many years?
Do you drink caffeinated drinks	❑ Yes ❑ No	If yes how much?	Type: ❑ Coffee ❑ Tea ❑ Soda
Do you normally eat a balanced diet	❑ Yes ❑ No	Meals per day?	Snacks per day?
Do you exercise on a regular basis?	❑ Yes ❑ No	Days per week?	Type of:

Have you had any of the following tests? If yes, include last year you had the test.

❑ EKG, Year _____ ❑ Stress Test, Year _____ ❑ Colonoscopy Year _____ ❑ Mammogram, Year _____

Check any symptoms and or conditions listed below that you have experienced in the **past 12 months**:	
Vision:	❑ Change in far vision ❑ Change in near vision ❑ Blurred Vision
Hearing:	❑ Ear pain ❑ Loss of Hearing ❑ Ringing in Ears
Musculoskeletal	❑ Joint Pain ❑ Joint Stiffness ❑ Muscle weakness ❑ Unsteady Walking
Cardiovascular	❑ Chest pain ❑ Palpitations
Respiratory:	❑ Shortness of breath ❑ Wheezing ❑ Coughing ❑ Coughing up blood
Circulatory:	❑ Swelling of the Hands/Feet ❑ Leg Cramps with walking
Endocrine:	❑ Excessive thirst ❑ Frequent urination ❑ Unintentional Weight Change > 5 lb.
Gastrointestinal	❑ Diarrhea ❑ Constipation ❑ Blood in stools ❑ Heartburn
Neurological	❑ Headaches ❑ Numbness or tingling in extremities
Emotional:	❑ Depression ❑ Anxiety

I certify that this information is correct to the best of my knowledge.

X

Participants Signature:	Date:
Reviewed by Clinician's signature:	Date:

Appendix C

FOOD RECORD

INSTRUCTIONS FOR KEEPING YOUR 3 DAY DIET RECORD

The purpose of this diet record is to assess your normal food and beverage intake. Please do not change your normal diet as it is important for us to know what you really eat. Below are specific recommendations on how to most accurately record your food and beverage consumption as well as tips on portion sizes.

I. Details on recording food intake:

1. Use the provided food log sheets to write down everything you eat and drink for the three days you have chosen. Please avoid holidays, birthdays, party days, or any day that is out of the ordinary.
2. Two of the days documented should be from Monday through Thursday. One of the days should be a Saturday or Sunday.
3. Include all the beverages you consume, including alcohol and water.
4. Be sure to include all sauces, gravies, dressings, cream and sugar for coffee, etc., as these items contribute to your total calorie intake.
5. Describe how the food was prepared (fried, boiled, baked, etc) and how it was served (with cream sauce, Italian dressing, etc).
6. Estimate as closely as you can the portion size you consumed. Some examples of typical portion sizes can be found on the next page.
7. To be as accurate as possible, it is best to carry this food record around with you and write down what you eat and drink soon after your meal, rather than trying to remember what you ate several days later.
8. If you eat in a fast food restaurant, write down the place as well as the foods you ate, as specific brand names can help us in our analysis of your diet.

Estimating Portion sizes

Fruits and Vegetables

- 1 c of fruit or vegetable = a baseball
- 1 medium sized fruit = a tennis ball
- ¼ c dried fruit = a golf ball
- 2-inch slice of melon = width of 3 fingers
- Medium potato = size of a computer mouse

Meats, nuts, and other protein rich foods

- 3 oz meat/poultry/fish = a deck of cards
- 1 oz nuts= About one handful
- 2 Tbs peanut butter = a marshmallow or a golf ball

Dairy

- 1 ounce cheese = 4 dice or about the size of your thumb
- 1 ½ oz cheese = 6 stacked dice
- ½ c ice cream = a racquetball

Breads and grains

- ½ bagel = small soft drink lid
- ½ cup cooked cereal = small fist or ½ of a baseball
- 1 pancake or waffle = music CD
- 2 oz chips or pretzels = about two handfuls
- 1 cup of pasta = tennis ball
- 1 tortilla = small (7 inch) plate

Fats

- 1 teaspoon margarine or butter = thumb tip
- 2 tablespoons butter = golf ball
- 1 tablespoon salad dressing = ping-pong ball

Desserts

- 1 oz small candies (ie. jellybeans): About one handful
- 4 small cookies (like vanilla wafers) = four checkers or poker chips

FOOD INTAKE LOG

Day of the Week:_____

Meal/Time of Day	Food/Drink (specify brand or restaurant name)	Amount

FOOD INTAKE LOG

Day of the Week:_____

Meal/Time of Day	Food/Drink (specify brand or restaurant name)	Amount

FOOD INTAKE LOG

Day of the Week:_____

Meal/Time of Day	Food/Drink (specify brand or restaurant name)	Amount

SUPPLEMENT INFORMATION

Please record the names of the supplements you take (include Brand name) as well as thedosage, the number of times you take it, and what it contains.

Supplement 1: _____
Dose: _____
How often do you take it: _____
What does it contain:

Supplement 2: _____
Dose: _____
How often do you take it: _____
What does it contain:

Supplement 3: _____
Dose: _____
How often do you take it: _____
What does it contain:

Supplement 4: _____
Dose: _____
How often do you take it: _____
What does it contain:

Appendix D

INFORMED CONSENT

Research Study: **The Relationship between Habitual Salt and Potassium Intake on Vascular Function**

Investigators: Shannon Lennon-Edwards, PhD, RD; David G. Edwards, PhD; William Farquhar, PhD, Brittany Allman, BS; Jennifer DuPont, MS; Evan Matthews, MS

Subject Name_____

1. PURPOSE / DESCRIPTION OF THE RESEARCH

You are being asked to participate in a research study conducted by the Department of Behavioral Health and Nutrition and the Department of Kinesiology and Applied Physiology at the University of Delaware. The purpose of this research is to determine the effects of salt and potassium intake on blood vessel function.

You will be one of approximately 80 participants. We are recruiting both men and women aged 18-40 years and 50-75 years.

Full participation in this study will require 2 visits to the Vascular Physiology Lab in 223 McDowell Hall, 25 N. College Avenue in Newark, DE 19716. During visit 2, you will also go to the Nurse Managed Health Center in room 119 of McDowell Hall.
Total time commitment for this study is approximately 6 hours.

The time commitment in the lab is approximately 2 hours.

Visit 1 will take 30-45 minutes.

Visit 2 will take 1 ½ hours.

The time commitment outside of the lab visits will total about 4 hours.

2. WHAT YOU WILL DO

VISIT 1:
Your first visit to the Vascular Physiology Lab in 223 McDowell Hall will last approximately 30-45 minutes.
The following will occur:

- You will complete a medical history questionnaire that asks about your current and past health as well as a questionnaire on your exercise habits.

- You will receive instructions on how to record your food and beverage intake for 3 days.
- You will receive instructions on how to collect your urine for 24 hours prior to your second visit to the McDowell Hall.
- You will receive instructions on how to use the Actical accelerometer on the 3 days you select to record your food and beverage intake.
- Resting heart rate, blood pressure, height, and weight will then be measured.

What you will do between Visit 1 and 2 at home:

- Select 3 days to record your food and drink intake. Two days should be a weekday (Mon through Thurs) and one day should be a weekend (Saturday or Sunday). The third day you select should be the day prior to your scheduled visit 2 to the McDowell Hall.
- You must collect your urine on the third day you record your food and beverage intake and bring the urine collection to your visit 2 at the McDowell Hall.
- You will wear the Actical accelerometer on all 3 days you record your food and beverage intake. The accelerometer will keep track of your physical activity. You will wear the device on your hip and will only take it off to shower and to sleep.

VISIT 2:

Your second visit to the Vascular Physiology Lab will last approximately 1 ½ hours. Prior to this visit you will be asked not to eat food for 12 hours, drink alcohol or caffeine for 12 hours, and to not exercise for 24 hours.
For this visit, you will be asked to bring a t-shirt and shorts, your 3 day food record, the Actical accelerometer, and your 24 hour urine collection.

- Your diet record will be analyzed for its average calorie intake as well the amount of nutrients you consume on an average day.
- Your urine will be analyzed for sodium and potassium excretion (e.g. electrolytes) and kidney function. The volume of urine will also be recorded.

<u>The following will occur:</u>

- You will first go to the Nurse Managed Health Center on the first floor of McDowell Hall (room 119) to have your blood drawn by a Nurse Practitioner and they will do a quick review of your medical history. They will measure blood pressure and resting heart rate.
- The blood sample will be collected by inserting a needle into an arm vein (approximately 5 tablespoons or roughly a ¼ cup of blood is taken. This is much less than when you donate blood and they take 1 pint or roughly 2 cups).

- The blood sample will be used to make an assessment of your electrolytes, hemoglobin and hematocrit levels. This will be done in McDowell Hall.
- Some of your blood will also be stored (frozen) for future measurement of hormones that control salt and water balance in your body. Your blood sample will be coded but not de-identified. The information linking your name to the coded blood sample will remain in an encrypted file. With your permission, any remaining stored blood will be kept for future research studies regarding dietary salt and blood vessel function. After 5 years, all remaining blood will be disposed of following University of Delaware guidelines for disposal of blood.

- You will then go to the Vascular Physiology Lab and your weight will taken.

Assessment of blood vessel function:

- You will have three self-adhesive electrodes placed on your chest and you will then lie down and rest for 15 minutes in order to relax and make sure your heart rate is at resting levels.
- First, a pressure probe will then be placed over the artery in your wrist on top of your skin and recordings of pressure will be taken. This will be followed by a pressure recording of the arteries in your neck and groin (carotid and femoral arteries).
- A cloth tape measure will be used to measure the distance between the points where the recordings were made. This will be done by asking you to pull up your shorts to your underwear line.
- Second, a narrow blood pressure cuff will be placed on your forearm close to your elbow. Without the cuff inflated, ultrasound pictures will be taken on your upper arm (brachial artery). The cuff will then be inflated for 5 minutes. The sensation felt will be similar to having fallen asleep on your arm (slight numbness). The ultrasound pictures will be repeated for 2 minutes after cuff deflation as well.

2. CONDITIONS OF SUBJECT PARTICIPATION

Information obtained from this study will be kept strictly confidential. You will not be individually identified, except by a subject number known only to the investigators. All data will be stored on a password protected computer and the data will be encrypted. While the results of this research may be published, neither your name nor your identity will be revealed.

You are free to discontinue participation at any time without penalty.

In the event of injury during these research procedures, you will receive emergency first aid. If you require emergency room or other additional medical treatment, you will be responsible for the cost.

3. RISKS AND BENEFITS

There are no known risks associated with taking your height, weight, or resting blood pressure. You may have pain and/or bruising at the site where blood is taken, and there is a small risk of infection. Fainting sometimes occurs during or shortly after blood is drawn.

The ultrasound testing involves the inflation of a blood pressure cuff which may result in some temporary mild discomfort similar to that experienced if your arm went numb if you fell asleep on it.

There may be no direct benefit to you for participating in this research study however you will be provided the results of the analysis of your diet when you complete the study. These results will not be interpreted but are provided for your information.
There is no compensation for participation in this study.

4. FINANCIAL CONSIDERATION

There is a $30 compensation for participation.

5. CONTACTS

Any questions regarding the study can be directed to:

Dr. Shannon Lennon-Edwards, PhD, RD, Assistant Professor, Department of Behavioral Health and Nutrition at (302)831-2798
Questions regarding the rights of individuals who agree to participate in this research may be directed to: Chair, Human Subject Review Board, University of Delaware at (302)-831-213

5. SUBJECT 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 stop my participation in this study at any time. By signing this form, I agree to take part in this research study and to allow the use of the described information for the purposes of research until the end of the study.

6. CONSENT SIGNATURES

_____Date:_____
Subject's Signature

_____
Subject's Name (Printed)

___ Yes ___ No Do we have your permission to contact you for future studies?

___ Yes ___ No Do we have your permission to keep your stored blood for future research studies?

Appendix E

INSTRUCTIONS FOR URINE COLLECTION

- You will be asked to collect **ALL** your urine during the day. For this 24-hour urine collection, you will begin by emptying your bladder at the laboratory and NOT collecting this…. so you will be starting with an 'empty' bladder.

- You will be given sheets which you can place in your bathroom(s) at home, and to keep with you in your office to remind you to collect all your urine in the container, and **NOT** to use the toilet.

Numbers to call if you have any comments, questions, or concerns (symptoms)

Shannon Lennon-Edwards, PhD 302-382-4291 (cell); 302-831-2798 (office)

Bill Farquhar, PhD 302-397-1591 (cell)

Questions about the Diet / Foods / Proper Actical Use

call BRITTANY: 484-802-6852

***Wear athletic shorts on final visit

***No caffeine or alcohol 12 hours prior to second visit

***No food 4 hours prior to second visit

***No exercise 24 hours prior to second visit

Appendix F

INSTRUCTIONS FOR ACTICAL® PHYSICAL ACTIVITY MONITOR

- You will be asked to wear the Actical for 24 consecutive hours. During this time, you may take off the device **ONLY** to shower and sleep.
- To begin recording activity, press the **STAR** button on the Actical.
- Device is to be worn on a Velcro belt (supplied) on the **right hip bone**.
- If you choose to exercise during this 24 hour period, you will be instructed to push the star button on the device to track your change in activity level.

Numbers to call if you have any comments, questions, or concerns (symptoms)

Shannon Lennon-Edwards, PhD 302-382-4291 (cell); 302-831-2798 (office)

Bill Farquhar, PhD 302-397-1591 (cell)

Questions about the Diet / Foods / Proper Actical Use

call BRITTANY: 484-802-6852

***Wear athletic shorts on final visit

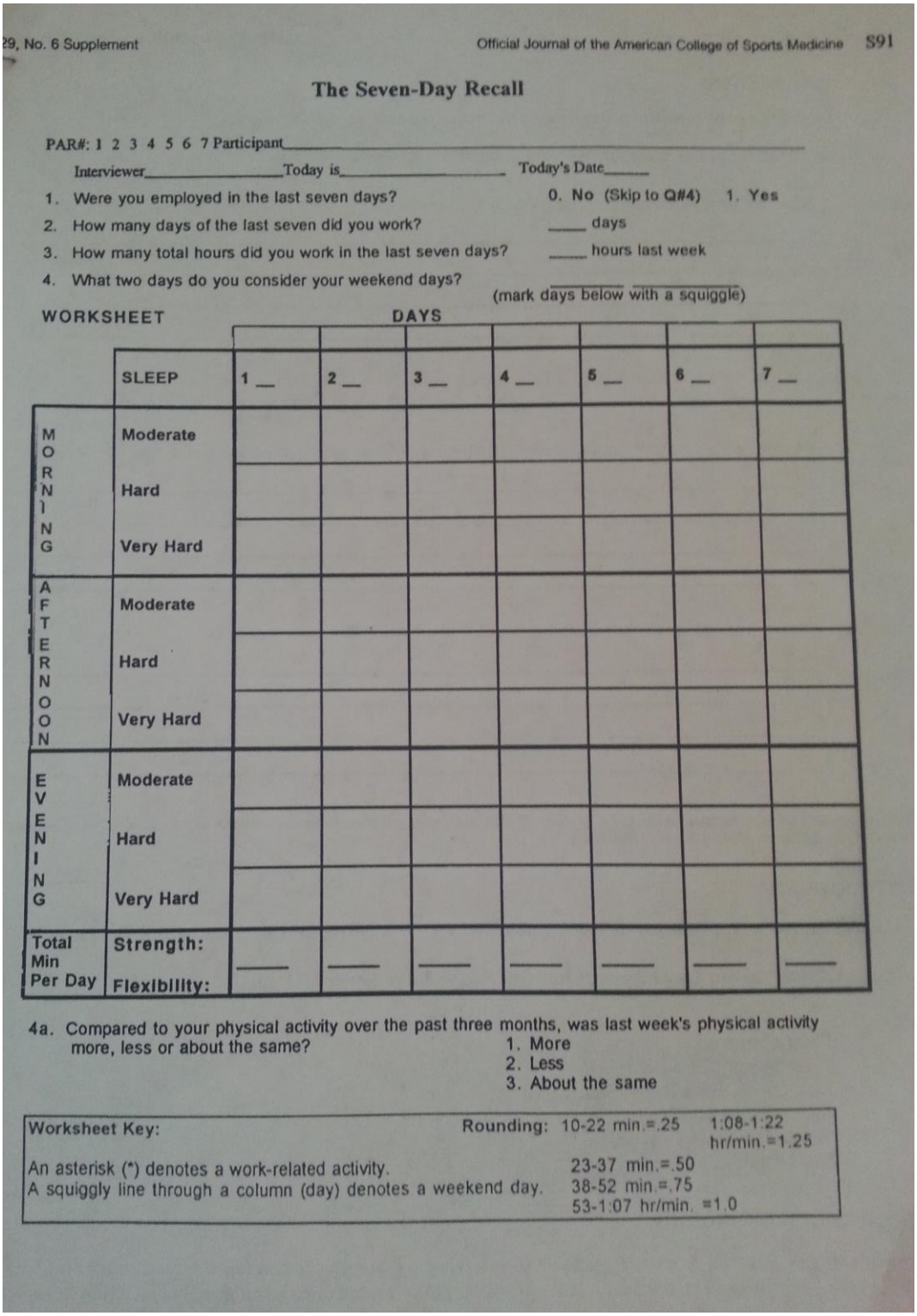
***No caffeine or alcohol 12 hours prior to second visit

***No food 4 hours prior to second visit

***No exercise 24 hours prior to second visit

Appendix G

PHYSICAL ACTIVITY QUESTIONAIRRE



The Seven-Day Recall

PAR#: 1 2 3 4 5 6 7 Participant_____

Interviewer_____Today is_____ Today's Date_____

1. Were you employed in the last seven days? 0. No (Skip to Q#4) 1. Yes
2. How many days of the last seven did you work? ____ days
3. How many total hours did you work in the last seven days? ____ hours last week
4. What two days do you consider your weekend days? _____ _____
   (mark days below with a squiggle)

WORKSHEET

		DAYS						
	SLEEP	1 __	2 __	3 __	4 __	5 __	6 __	7 __
MORNING	Moderate							
	Hard							
	Very Hard							
AFTERNOON	Moderate							
	Hard							
	Very Hard							
EVENING	Moderate							
	Hard							
	Very Hard							
Total Min Per Day	Strength:	____	____	____	____	____	____	____
	Flexibility:							

4a. Compared to your physical activity over the past three months, was last week's physical activity more, less or about the same?
1. More
2. Less
3. About the same

Worksheet Key:
An asterisk (*) denotes a work-related activity.
A squiggly line through a column (day) denotes a weekend day.

Rounding: 10-22 min.=.25 1:08-1:22 hr/min.=1.25
23-37 min.=.50
38-52 min.=.75
53-1:07 hr/min. =1.0

INTERVIEWER:

Please answer questions below and note any comments on interview.

5. Were there any problems with the 7-Day PAR interview? 0. No
   1. Yes (If yes, please explain.)

   Explain any problems you had with this interview:

   _____

   _____

   _____

   _____

6. Do you think this was a valid 7-Day PAR interview? 0. No
   1. Yes

7. Please list below any activities reported by the subject which you don't know how to classify.

   _____

   _____

   _____

   _____

8. Please provide any other comments you may have in the space below.

   _____

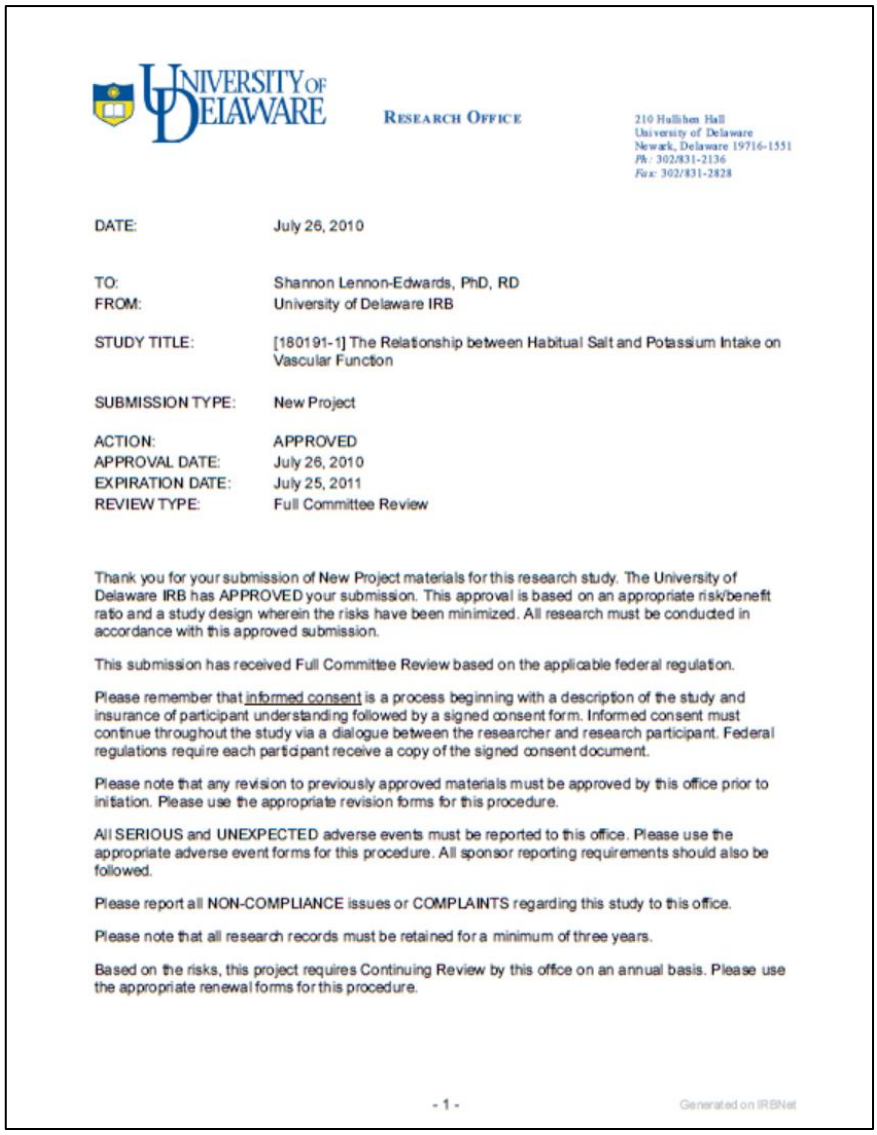
   _____

   _____

   _____

Appendix H

IRB HUMAN SUBJECTS APPROVAL



UNIVERSITY OF DELAWARE

RESEARCH OFFICE

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

DATE: July 26, 2010

TO: Shannon Lennon-Edwards, PhD, RD
FROM: University of Delaware IRB

STUDY TITLE: [180191-1] The Relationship between Habitual Salt and Potassium Intake on Vascular Function

SUBMISSION TYPE: New Project

ACTION: APPROVED
APPROVAL DATE: July 26, 2010
EXPIRATION DATE: July 25, 2011
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.

- 1 -

Generated on IRBNet

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

Appendix I

IRB HUMAN SUBJECTS APPROVAL AMENDMENT

UNIVERSITY OF DELAWARE

RESEARCH OFFICE

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

DATE:	February 11, 2013
TO:	Shannon Lennon-Edwards, PhD, RD
FROM:	University of Delaware IRB
STUDY TITLE:	[180191-9] The Relationship between Habitual Salt and Potassium Intake on Vascular Function
SUBMISSION TYPE:	Amendment/Modification
ACTION:	APPROVED
APPROVAL DATE:	February 11, 2013
EXPIRATION DATE:	July 25, 2013
REVIEW TYPE:	Expedited Review

Thank you for your submission of Amendment/Modification 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 Expedited 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.