THE RELATIONSHIP BETWEEN THE SODIUM/POTASSIUM RATIO AND VASCULAR FUNCTION

by

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ABSTRACT

Hypertension (HTN) is an extremely prevalent form of cardiovascular disease and currently afflicts 74.5 million Americans. Dysfunction of the vasculature may predispose an individual to developing HTN or high blood pressure (BP). Lifestyle factors such as diet and exercise play an important role in influencing BP. Research has shown that diets high in sodium can raise BP, whereas a high potassium diet may be protective against this. Further, evidence is mounting that a high sodium intake may be detrimental to the vasculature. The purpose of this study was to perform a cross-sectional examination of the role of sodium and potassium intake on vascular function in young healthy adults. Thirty-two subjects (16M, 16F) with an average age of 24 ± 3.4 years were recruited for this study. Participants recorded their dietary intake for 3 days and following the third day, underwent vascular function testing to determine pulse wave analysis (PWA), carotid-femoral pulse wave velocity (PWV), and flow-mediated dilation (FMD) of the brachial artery. Sodium and potassium excretion was measured using a 24-hour urine collection. Analysis of the dietary data indicates that subjects consumed above recommended levels of sodium (3862.15±293 mg/day) and below recommended levels of potassium (3922±148 mg/day) over the 3 days. Individuals with high sodium consumption had the highest sodium/potassium excretion ratio (p=0.024), and those with the highest potassium consumption had the
lowest sodium/potassium excretion ratios (p=0.04). For the males, a low sodium/potassium excretion ratio was associated with better augmentation index (p=0.031). This relationship did not exist for the females. Additionally, there was no relationship between sodium consumed nor sodium excreted and FMD or PWV in both males and females. Further, there was no relationship between potassium and these measurements. In conclusion, in this small sample size, no clear relationship between sodium intake and vascular function could be established for all subjects. In regards to sex, men with lower ratios exhibited more elastic arteries but more research is needed to explore this topic further.
Chapter 1

INTRODUCTION

Cardiovascular disease (CVD) is currently the number one cause of death in
the United States, with more than 2200 Americans dying from CVD each day\(^1\).
According to the most recent statistics from American Heart Association (AHA), the
overall rate of death attributable to CVD was 244.8 per 100,000 people, or
approximately one in six deaths in 2008. Often, these are recurrent events\(^1\).
Cardiovascular disease is associated with several major risk factors, including high
blood pressure (BP), dyslipidemia, obesity/overweight, diabetes mellitus, smoking,
and poor physical activity levels.\(^1\) Among individuals with CVD, 48% currently
smoke, 45% are hypertensive, 35% have dyslipidemia, 71% are obese or overweight,
17% are diabetic, 66% have no moderate or vigorous activity at all, and 70% meet 0 or
1 of the 5 healthy dietary measures\(^1\). Furthermore, the AHA statistics report that
approximately 74.5 million people in the United States age 20 and older have high BP.
This translates to one in three U.S. adults. High BP is also associated with a high
sodium intake, and reduced sodium intake is associated with lower BP\(^2\). Additionally,
high potassium intake is associated with low BP\(^3,4\). Because of this, it may be
beneficial to lower salt intake and increase potassium intake on a population wide
level\(^3-5\).
CHAPTER 2
REVIEW OF THE LITERATURE

2.1 Sodium

*Sodium in the Diet*

Until approximately 5,000 years ago, humans did not use salt in our diets. However, when salt was discovered as a means of preserving food, it was introduced into our diets, and quickly gained popularity causing the usage to soar. Adding salt to foods made otherwise unpalatable foods not only edible, but desirable. Individuals quickly began picking up the saltshaker and adding the new seasoning to their foods. The recommended amount of salt for individuals is 5-6 g/day (1,965-2,360 mg sodium/day (with an emphasized effort to lower this number further).

Sodium intake recommended in the 2010 Dietary Guidelines for Americans is less than 2,300 mg/day (or 5.84 grams of salt) for healthy, normotensive individuals, and less than 1,500 mg/day (or 3.8 grams of salt, which is about 2/3 of a teaspoon of table salt) for individuals more likely to be affected by a higher salt diet. Those individuals include African Americans, hypertensive individuals, and the elderly, who comprise approximately 70% of the U.S. population. Consequently, the Dietary Guidelines for Americans established these guidelines in order to appropriately counsel the population as a whole.

Several organizations recommend varied amounts of sodium intake, but these recommendations fall within the same general boundaries. For adults, the highest
limit proposed is 2,300 mg/day from the American Society of Hypertension and the National High Blood Pressure Education program, but both stress a lowered intake if possible\textsuperscript{7}. The AHA adheres to the same guidelines as the Dietary Guidelines for Americans, suggesting no more than 1,500 mg/day of sodium intake. International standards follow similar guidelines to the U.S., recommending that intake fall below 2,200 mg/day for individuals, and even less depending on whether an individual is hypertensive, black, or elderly.

Sources of High Salt

Salt within the diet may come from a variety of different sources, but the main three contributors are table salt, restaurant incorporated salt, and salt incorporated by manufacturers. Typically, 15\% of dietary salt comes from the table and/or additions in cooking, 5\% comes from natural sources, and 80\% comes from the food industry\textsuperscript{5,8}. Though salt was initially used as a form of preservation, it did not take individuals long to realize that salt could be used to drastically change the flavor of food at a very low cost. Foods that otherwise may have been unpalatable were now marketable, because masking the true flavor of the food with salt allowed individuals to enjoy the food more.

The main foods that are high in salt that the U.S. population consumes are yeasty breads, chicken and beef, pizza and pasta, cold cuts, condiments, sausages and franks, bacon and rib meat, cheeses, dessert foods made with a grain base, and finally, soups. These foods account for nearly 2000 mg/day of sodium consumption in the U.S.\textsuperscript{7}. Sodium consumption is also directly related to energy intake; individuals who
are over eating are also consuming significantly more sodium than they should be. By cutting down on excess eating, sodium intake should also be naturally lowered\(^7\). It is unlikely that cutting calories alone would cause sodium consumption to fall below at or below the recommended intake, but it would certainly be a start in terms of sodium reduction.

*Why is Salt bad for us?*

High levels of salt within the body are harmful for many reasons. High salt intake in the diet is associated with high BP. As levels of salt increase in the body, circulatory volume rises, which in turn increases the excess fluid pressure on the blood vessel walls. One result of this process can be thickening of the vessel walls and a narrower vessel diameter, leaving less space for the blood within the vessel. This reduction in diameter causes an increased peripheral resistance and subsequently a higher pressure requirement to move blood to the organs in the body. Therefore, BP is raised because the heart has to pump against a greater peripheral resistance. In some individuals, this can cause the heart to enlarge and lead to heart failure (HF). Notably, hypertension is seen in 75% of those individuals with diagnosed HF making it one of the most significant risk factors.

The kidneys are essential in maintaining fluid and electrolyte balance within the body. The kidneys maintain this balance by regulating the amount of water excreted in the urine, as well as regulating plasma ionic composition, which includes sodium and potassium. Because of their role in water excretion, the kidneys also have an effect on BP. Plasma volume is controlled by how much water is excreted, which
has a direct effect on total blood volume, and therefore BP\textsuperscript{9}. Antidiuretic hormone (ADH) controls the water excretion in the kidneys by resorbing or excreting water to maintain fluid balance. There are a number of factors that influence ADH, including concentrated blood plasma as well as low BP\textsuperscript{9}.

The kidneys regulate salt balance by the release of aldosterone, which helps control arterial pressure. Aldosterone increases the rate of sodium and water reabsorption, which consequentially reduces the loss of sodium and water in the urine, thus increasing blood volume and extracellular fluid. Additionally, aldosterone promotes the excretion of potassium.

The renin-angiotensin vasoconstrictor mechanism is also important for controlling arterial pressure. In general terms, the higher the amount of sodium an individual ingests, the more sodium that must be excreted to keep the body’s fluids in balance\textsuperscript{10,11}. The increase in osmolality caused by the excess salt will stimulate release of antidiuretic hormone causing the kidneys to reabsorb water. If an individual consumes high levels of sodium regularly, their BP may rise, creating the high-pressure system described previously. The increased pressure results in a decrease in renin and angiotensin. Renin is decreased in high-pressure situations because it is a protein enzyme that helps raise arterial pressure; decreasing renin helps to decrease pressure. Angiotensin acts directly on the kidneys to cause salt and water retention, so in a high pressure situation, suppressing angiotensin also results in a lowered arterial pressure. Because of the decrease in renin and angiotensin, retention of salt and water
is also decreased, which helps return extracellular volumes to near normal. Finally, the arterial pressure returns to normal\textsuperscript{11-13}.

In individuals who are hypertensive, angiotensin and aldosterone levels are increased. Angiotensin is converted to angiotensin II, which can elevate arterial pressure by causing vasoconstriction and a decreased excretion of both sodium and water by the kidneys. The increased aldosterone levels result in increased sodium and water reabsorption thus resulting in an increased arterial pressure. The direct effect of angiotensin and aldosterone on the kidney are important in terms of long-term arterial pressure control and in the instance of high BP in individuals with hypertension\textsuperscript{9}.

Onset of hypertension occurs predominantly for individuals 50 years and older, Currently, 33.5\% of Americans are hypertensive, and another third of the population is pre-hypertensive\textsuperscript{1,13,14}. Because BP increases as we age, individuals 50 years and older have a 90\% likelihood of becoming hypertensive\textsuperscript{15} but younger individuals are becoming increasingly affected, as well\textsuperscript{16}. Pre-hypertensive individuals are at higher risk of developing HTN according to a report on heart disease and stroke released by Circulation in 2012\textsuperscript{1}. Currently, 30\% of individuals 20 years and older are pre-hypertensive. Looking more specifically at a younger population, 28\% of individuals 20-34 years old are prehypertensive\textsuperscript{16}. The AHA categorizes prevalence of HTN by age. Specifically, 11.1\% of males and 6.8\% of females 20-34 years old are hypertensive. Further, 25.1\% of males and 19.0\% of females aged 35-44 years old hypertensive. Taking this data combined, 36.2\% of males and 25.8\% of females 20-44 years old living in the United States are hypertensive. That being said, high salt intake
appears to play a role in HTN for individuals as young as 20 years old. This intake may also play a role in pre-hypertension in individuals who are in their 40s and older.

As stated, our current salt intake is 9-12 g/day, with a push for individuals to lower intake to 5-6 g/day. Studies have shown that reducing salt intake to 5-6 g/day has the same effect as taking one BP lowering medication\textsuperscript{17}. However, individuals on BP lowering medication were not the only ones who benefited from lowering their salt intake. Those individuals who were not currently taking BP lowering medications also experienced a significant decrease in their BP\textsuperscript{17}. Additionally, in an analysis of salt reduction trials, it was found that a reduction of salt to 6 g/day could reduce the instance of strokes by 24% and the instance of coronary heart disease by 18%\textsuperscript{17}. As previously stated, CVD is the cause of 62% of strokes and 49% of CHD\textsuperscript{18}. Ultimately reducing salt intake would prevent approximately 2.5 million CHD related deaths on a global level\textsuperscript{19}. A study performed by Tuomilehto et al.\textsuperscript{20} found that an increased consumption of 6 g/day of salt above usual intake was related to a 56% increase in CHD death, as well as a 36% increase in CVD related deaths, and 22% increase in death of any kind. These studies help demonstrate that increasing salt intake has a damaging effect much like reducing salt intake has a very positive effect. In addition, Page et al.\textsuperscript{21} surveyed a population that had no access to manufactured salt, and the individuals within the population had a significantly lower BP.
2.2 Potassium

Potassium in the Diet

Potassium is an important component of the diet. It is a mineral, found most abundantly in potatoes, soymilk, white beans, avocados, fish, and many fruits and vegetables. Despite the wide variety of foods rich in potassium, most individuals do not consume enough daily. According to recent surveys, men of all ages are consuming 3,200 mg/day while women are consuming 2,400 mg/day. The AI for potassium (as released in the Dietary Guidelines for Americans 2010) is 4,700 mg/day for both men and women. Because potassium is readily excreted in the urine, there is no major risk factor associated with overconsumption of potassium for healthy individuals. As stated previously, potassium is available in fruits and vegetables, but most individuals consume the majority of their potassium in reduced fat milk, coffee, chicken, beef, and grapefruit and orange juice. High energy intake is highly associated with potassium consumption; target potassium levels are generally met with individuals consuming 3,000-3,200 calories/day. However, individuals are recommended and encouraged to find a healthy way to incorporate more potassium into their diet without consuming too many calories.

Why is Potassium good for us?

Both potassium supplements and potassium within the diet have a positive health related effect for individuals. By increasing the level of potassium intake to the recommended amount (4,700 mg/day), individuals may be able to lower their BP. Few studies exist that have analyzed the effect of potassium consumption on diet, but of
those that do, all have found a significant reduction in BP in relation to increased potassium intake. In a study done by Morris et al., 38 normotensive, healthy black and white men were instructed to eat a low sodium diet (15 mmol/day) deficient in potassium (30 mmol/day) for six weeks. During the final four weeks, NaCl was loaded (250 mmol/day) and for the final three weeks, potassium was supplemented at either 70 or 120 mmol/day. While consuming a low potassium diet, salt loading induced a mean increase in BP only in blacks. Further, the salt sensitivity occurred in most blacks (79%) but not as many whites (36%) in this study. Supplementation of potassium at 70 mmol/day attenuated salt sensitivity in blacks and whites, and supplementing potassium at 120 mmol/day completely abolished salt sensitivity for the men, suggesting that potassium may play a role in salt sensitivity and therefore BP.

In another study by Morgan et al, male patients with high BP were supplemented with potassium chloride (48 mmol/day). High sodium intake individuals receiving the supplement had a significant decrease in both systolic and diastolic BP.

Of the studies that consider the effect of potassium intake on BP, few differentiate between supplemental potassium use and potassium within the diet. In a study performed by Appel et al., individuals 22 years and older with a systolic BP <160 mm Hg and a diastolic BP of 80-95 mmHg were recruited for a diet intervention study. For three weeks, individuals were fed a control diet low in fruits, vegetables, and dairy products and a fat content typical of the American diet. After three weeks on the control diet, subjects were randomly assigned to consume eight weeks on the same control diet, eight weeks on a diet rich in fruits and vegetables, or eight weeks of a
combination diet rich in fruits, vegetables, and low-fat dairy products and low in saturated and total fat. The combination diet served as the DASH (Dietary Approached to Stop Hypertension) diet. After the eight weeks of diet consumption, individuals on the combination diet had reduced their systolic BP by 5.5 mmHg and diastolic BP by 3.0 mmHg more than individuals on the control diet (p<0.001 for each). The fruit and vegetable diet also reduced BP compared to the control diet, but not by as much (2.8 mmHg reduction for systolic BP and 1.1 mm Hg reduction for diastolic BP; p<0.001 and p=0.07, respectively).23

In addition to its positive effects on lowering BP, potassium within the diet can also alleviate the negative effects that high sodium intake has on BP. These effects have been documented by various trials. An interesting outcome of these trials is that high potassium intake has the greatest effect on black non-hypertensive individuals and hypertensive individuals3,4,24. Given this information, the implications for what increased intake of potassium could do for both normotensive and hypertensive/prehypertensive individuals are intriguing and very encouraging.

2.3 Sodium/Potassium Ratio

This section will review a number of studies that are related to the sodium/potassium ratio and the association with CVD. There are a number of studies that consider the interaction effect of this ratio, whether it is in terms of consumption or excretion values.
INTERSALT

The INTERSALT study was undertaken in 32 different countries, with 10,079 men and women between the ages of 20 and 59 years old participating. The purpose of this study was to look at 24-hour urinary sodium excretion and it’s relationship with BP. After multivariate adjustment for reliability and controlling for age, sex, alcohol intake, and potassium, this study found that a 100 mmol/24-hour increased sodium intake resulted in a higher systolic BP by 3-6 mmHg and a higher diastolic BP by 0-3 mmHg\textsuperscript{25}. This relationship existed regardless of current hypertension status. This study was novel in that it was a worldwide epidemiological study that looked at 24-hr sodium excretion and BP. Ultimately, the data from this study correspond to findings from other studies, including clinical observation, therapeutic interventions, animal studies, control trials, and epidemiological experiments\textsuperscript{2,11,25-27}. All of these investigations support the notion that habitual high sodium intake is one of the most measureable and important factors causing population-wide high BP, which is a major risk factor for CVD\textsuperscript{11,25-27}.

Dietary Approaches to stop Hypertension (DASH)

The DASH diet is a diet that has been formulated to help pre-hypertensive and hypertensive individuals lower their BP. This diet recommends specific consumption of fruits, vegetables, plant based proteins, and low-fat dairy products. With a 2,000 calorie/day diet\textsuperscript{28}, recommendations include 7-8 servings of whole grains, 4-5 servings of vegetables, 4-5 servings of fruits, 2-3 servings of nonfat dairy a day. Eating a
DASH regulated diet as well as limiting salt intake has the greatest effect on lowering BP.

In a study done by Sacks et al., 26 421 individuals (mean age 48 years) who were either pre-hypertensive, or stage 1 hypertensive were assigned to an intervention diet. Researchers created 3 different diets (high salt ~150mmol/day, intermediate salt ~100 mmol/day, and low salt ~50mmol/day). From there, researchers created two different diets: one was typical of the average American diet (control diet), and one followed the restrictions for the DASH diet. There were a total of six different intervention diets. Researchers found that reducing the sodium intake from high to intermediate salt reduced SBP by 2.1 mmHg (p<0.001) for the control diet, and by 1.3 mmHg (p=0.03) for the DASH diet. Reducing sodium from intermediate to low level reduced SBP by 4.6 mmHg for the control diet (p<0.001) and 1.7mmHg for the DASH diet (p<0.01). The DASH diet was associated with a significantly lower SBP at each sodium level compared to the control diet. Compared to the high sodium control diet, the low sodium DASH diet had a mean SBP 7.1mmHg lower in participants with pre-hypertension, and 11.5mmHg lower in participants with hypertension 26. Past studies have indicated that there is a positive correlation between following the DASH diet and lowered BP and development of heart failure.

The Trials of Hypertension Prevention (TOHP) were studies focused on individuals 30-54 years old with hypertension 29. TOHP I tested the feasibility of seven non-pharmacological interventions in reducing BP for individuals with pre-hypertension 30. These interventions included weight loss, sodium reduction, stress
management, and supplementation of fish oil, calcium, magnesium, or potassium. The sodium reduction intervention involved dietary and behavioral counseling on how to identify sodium in the diet and prepare low-sodium meals, as well as weekly group counseling sessions. Participants in the control group received general healthy guidelines for healthy eating as their intervention. After an 18-month follow up, researchers found a net decrease in sodium excretion of 44mmol/24 hour, reduction of SBP by 1.7 mmHg, and reduction of DBP of 0.8mmHg (p<0.01 and p<0.05, respectively).

In TOHP II, the effects of weight loss and sodium reduction on BP over a 3-4 year follow-up were studied. Interventions were sodium reduction, weight loss, sodium reduction and weight loss, and usual care. Again, participants were 30-54 years old. They were also 110-165% of desirable weight, and were either pre-hypertensive or stage 1 hypertensive. Counseling sessions were similar to those in TOHP I. At 36 months, the sodium reduction group had a decreased SBP of 1.2mmHg (p=0.02) compared to the usual care intervention. The sodium reduction group also had a lower incidence of hypertension compared to usual care (p=0.05).

In a study done by Cook et al., researchers used data gathered in TOHP I and TOHP II to describe the relationship of usual long-term mean urinary sodium excretion with CVD, as well as the sodium/potassium urinary excretion ratio. For the follow-up study done by Cook et al., researchers used the data for the 2275 participants who were not assigned to a dietary intervention group in the TOHP study. Three to seven 24-hr urinary excretions for each individual were assessed to
determined sodium and potassium excretion, and the ratio was then compared to CVD events in 10-15 years post-trial follow up. Researchers found that there was a significant trend in CVD risk across quartiles of sodium/potassium excretion ratio, suggesting that a higher sodium/potassium excretion ratio is associated with an increased risk of subsequent CVD. This effect was stronger than either sodium or potassium alone.\textsuperscript{32}

O’Donnell et al.\textsuperscript{33} performed a similar follow-up analysis on a separate cohort to determine the effects of urinary sodium and potassium excretion and risk of CVD. This study was different than that done by Cook et al. in that it was an observation of individuals with CV disease or Diabetes Mellitus (DM). Sodium and potassium excretion were determined using an estimated 24-hour urine based on a fasted morning urine sample. This study included individuals from two cohorts: the ONTARGET and TRANSCEND trials. The main outcome measures assessed were CV death, MI, stroke, and CHF. This study separated individuals into groups based on sodium excretion of $<2$ g/day and $2-3.99$ g/day (low sodium excretion), $4-5.99$ g/day (baseline sodium excretion), and $6-8$ g/day (high sodium excretion). Researchers found that compared with baseline sodium excretion, high sodium excretion and low potassium excretion were associated with an increased risk of CV death, MI, stroke, and hospitalization of CHF. When plotted, these results represented a J-shaped curve. That is, in terms of potassium excretion, individuals with a potassium excretion less than $1.5$ g/day had a reduced risk of stroke compared to those individuals consuming $1.5-3.0$ g/day of potassium. Ultimately, researchers concluded that sodium excretion
of >7g/day was associated with an increased risk for CV disease, and sodium excretion less than 3g/day was also associated with increased risk of CV mortality and CHF. However, it is important to note that this study included only individuals with CV disease or DM, and the increased mortality in the low excretion groups was confined to the CV causes of death, and had no effect on non-CV death. This study was unique in that it considered the effects of sodium and potassium excretion in a population with CV disease and DM.33

The combined effect of sodium and potassium on cardiovascular health is also important to consider. The current literature suggests that a low sodium/potassium excretion ratio may have cardioprotective effects23,25,32. This is thought to be due, in part, to the protective action of the increased dietary potassium intake, which diminishes the mortality rate associated with high salt intake. This protective effect of potassium on sodium consumption has a greater effect than just sodium or potassium alone32. When there is a higher intake of potassium in the diet, the increased potassium results in increased sodium excretion.

**2.4 Cardiovascular Health**

Cardiovascular health is a very important aspect of individual health. The human cardiovascular system is a closed system in which oxygen and nutrients are carried through the blood and delivered to target cells. The cardiovascular system also carries carbon dioxide and waste products away from target tissues. Importantly, BP is controlled based on constriction and dilation of the blood vessel walls.
When the muscular wall of the blood vessel contracts, the blood vessel is said to have vasoconstricted. Blood flow is decreased during vasoconstriction which increases vascular resistance. Vasoconstriction is a mechanism used by the body to maintain mean arterial pressure (MAP). Molecules known as vasopressors, which include endothelin and angiotensin II, can initiate vasoconstriction.

Vasodilation is the widening of blood vessels, which is a result of smooth muscle relaxation within the vessel walls. When the blood vessels dilate, blood flow increases because of the decreased vascular resistance. The arterials are therefore dilated, resulting in a lowered BP. Vasodilators are the molecules that influence vasodilation. Vasodilation effects MAP, cardiac output (CO), and total peripheral resistance (TPR). Vasodilation helps to decrease TPR and BP by relaxing the smooth muscle cells in the arteries and arterioles.

Vasodilation and vasoconstriction are both mediated by paracrine agents from the endothelial cells. These include nitric oxide (NO), bradykinin, potassium ions, and adenosine.

There have been a handful of studies done in human populations determining the effects of salt intake on vascular function in individuals. A study by Jablonski et al. considered the association of low dietary sodium intake and endothelial function in middle-aged and older adults. Using brachial artery FMD as their measure of vascular function, researchers found that FMD was 42%-52% higher in the low sodium intake group verses the normal sodium group (p<0.05). In all subjects (regardless of sodium consumption level), brachial artery FMD was inversely related
to sodium intake (p<0.05). Finally, brachial artery FMD was not related to any other variables, suggesting that low sodium intake is associated with brachial artery FMD in both middle age and older adults. These results suggest that dietary salt intake plays a role in determining arterial stiffness for all individuals, whether they are pre-hypertensive, hypertensive, or healthy.

Dickinson et al. assessed vascular function as it relates to sodium in the diet as well as salt loading. In a randomized crossover trial, Dickinson et al. compared the effects of a low-salt meal and a high-salt meal on FMD. Individuals were fairly young (mean age 37.4 y/o), normotensive, and healthy (mean BMI of 24.8 kg/m²). The low-salt meal was 130mg of sodium, while the high-salt meal was 1,494 mg of sodium. After the completion of the meal, FMD was measured every 30 minutes for two hours. Researchers found that FMD 30 minutes after consumption of the low-salt meal was significantly higher than it was following the high-salt meal (6.05 ± 3.21 % compared to 3.39 ± 2.44%; p<0.01). The difference in FMD remained significant after an hour, as well (4.64 ± 2.48% compared to 2.20 ± 2.77%; p< 0.01). These findings show that a high-salt meal can significantly suppress brachial artery FMD, which suggests that high salt intakes have an acute adverse effect on the vasculature.

A random crossover trial done by Dickinson et al. assessed the effect of chronic salt reduction on vascular function. In this study, 29 normotensive overweight and obese individuals (mean age of 52.7 ± 6.0 years and BMI of 31.6 ± 2.8 kg/m²) were assigned to either a low-salt diet (730.4 ± 343.5 mg/day) or a usual-salt diet
(2048.4 ± 647.7 mg/day) for two weeks. After the two weeks, the percent change in brachial artery FMD was assessed (following an overnight fast) for each subject. Researchers found that FMD was significantly greater with the low-salt diet compared to the usual-salt diet (4.89 ± 2.42% compared to 3.37 ± 2.10%; p= 0.001). These results indicate that salt reduction can improve endothelial-dependent vasodilation in normotensive subjects. Therefore, the cardioprotective effects of a low-salt diet may exist beyond BP reduction and include the vasculature.\textsuperscript{36}

\textit{Endothelial Function}

A healthy endothelium controls local arterial tone by producing relaxation factors\textsuperscript{38}. The endothelial cells are the layer of cells that line the blood vessels, which reduce blood flow turbulence, affecting the distance of the blood flow and also helping to control BP. Endothelial cells are involved in vasoconstriction and vasodilation, and therefore help to control BP.

A healthy endothelium also has anticoagulant and anti-inflammatory properties. The endothelial cells directly affect the constriction and dilation of the vascular smooth muscle surrounding them. The endothelium promotes vasodilation by releasing vasodilators including NO and prostacyclin\textsuperscript{39}. NO was originally known as endothelium-derived relaxing factor (EDRF) because of its dilatory properties\textsuperscript{34}. An endothelium that functions correctly has the ability to release NO only as necessary to promote vasodilation. Many different vascular diseases are associated with endothelial dysfunction.
One of the mechanisms resulting in endothelial dysfunction is excessive production of reactive oxygen species (ROS). ROS are highly reactive molecules containing oxygen\textsuperscript{39}. There are a number of mechanisms where ROS affects NO bioavailability and synthesis. Peroxynitrite is an oxidant that is produced when superoxide and NO react, which is likely a cause of endothelial dysfunction due to the increased superoxide production. A second mechanism where ROS modifies NO bioavailability is when hydrogen peroxide and myeloperoxidase react. ROS can also oxidize tetrahydrobioterin, which aids in NO production, leading to endothelial dysfunction. A final mechanism where ROS can interfere with endothelial function is when it alters the actions of NO on vascular smooth muscle\textsuperscript{39}.

When a patient has abnormally high BP, vasodilators are used to dilate the blood vessels, therefore reducing BP. Damage to the endothelium upsets the equilibrium between vasodilation and vasoconstriction, which induce a series of events leading to atherosclerosis. Atherosclerosis causes an inflammatory response and subsequently the arterial walls are hardened and made stiffer. This stiffness in turn affects BP. Arterial stiffness does occur with aging but is increased by hypertension as well\textsuperscript{40}.

As indicated in the previous literature, a high salt diet negatively affects endothelial function, often resulting in cardiovascular disease and even mortality. High salt consumption causes the arterials to stiffen\textsuperscript{3}. The mechanism by which this happens is in depth, involving both TGF-\(\beta\) (Transforming Growth Factor Beta) and
NO. Salt influences the amount of both TGF-β and NO released into the body, thus affecting BP, heart health, and renal function.

Recent research in rats has found that a high-salt diet for nine months decreased the survival rate of rats in a dose dependent fashion by accelerating arteriosclerosis, arteriolosclerosis, and renal parenchymal damage. This acceleration is due to changes in the renin-angiotensin-aldosterone-system (RAAS) in response to high salt diets. Pathological changes in the RAAS due to high salt diets promote salt sensitivity, which weakens the ability for the cardiovascular and renal systems to adapt. Additionally, it has been found that rats fed a high salt diet (7% NaCl) showed an increased generation of ROS (compared to those rats fed a low-salt diet of 0.4% NaCl), which may be responsible for decreased endothelium-dependent responses.

**Nitric Oxide Production**

Nitric Oxide is a biological messenger in mammals. In the endothelium of blood vessels, NO signals the smooth muscle to relax, causing vasodilation and an increased blood flow. NO is highly reactive and therefore causes quick vasodilation.

A number of studies have shown that increased salt intake increases NO production. In a study on salt sensitivity, Sanders et al. looked at Sprague-Dawley young and old rats that were either susceptible or sensitive to the hypertensive effects of a high salt (8.0% NaCl) diet. While on the 8.0% NaCl diet, the salt sensitive rats rapidly developed hypertension and nearly all died at 8 weeks of age while the high salt diet only had a marginal effect on blood pressure in the salt resistant rats. The salt sensitive young rats fed a high-salt diet grew normally for 2-4 weeks, but as their
hypertension became more severe, they stopped growing, began losing weight, and then died. This study shows that there is a highly significant effect of a high salt diet on both young and older individuals, particularly concerning salt sensitivity\textsuperscript{47}.

Translating this study to humans would suggest that young salt sensitive individuals may also be negatively affected by a high salt intake. The endothelium is the source of increased NO production in rats fed a high-salt diet\textsuperscript{48}. The increased NO production facilitates increased salt excretion from the body. NO inhibition in high-salt diets has resulted in salt retention and salt-sensitive hypertension\textsuperscript{45,49}. Continued inhibition of NO can also contribute to renal function damage.

Production and release of NO and TGF\(\beta\)-1 are interrelated functions in the body. NO helps moderate TGF\(\beta\)-1 by limiting its production. Therefore, if there are low levels of NO in the system, then the levels of TGF\(\beta\)-1 production are increased. This increased level of TGF\(\beta\)-1 production could result in renal failure and heart problems\textsuperscript{41}. 
CHAPTER 3

SPECIFIC AIMS AND HYPOTHESIS

The role of increased dietary salt consumption on cardiovascular health has highlighted the association between dietary sodium and high BP. This work has also extended to suggest that high dietary sodium is deleterious to the vasculature\textsuperscript{29}. Additionally, research has shown that potassium can be protective in the presence of high salt intake\textsuperscript{32}. However, there are fewer studies that have focused on potassium intake and its effects on vascular health. Does potassium play a protective role in the vasculature prior to changes in BP? In order to evaluate the relationship between sodium and potassium intake on the vasculature, research suggests that the sodium to potassium excretion ratio is a critical factor to assess one’s vascular and hence, cardiovascular health. To date, there is not much research that has looked at this relationship. Those that have do not use the 24-hour urinary recall method, which is most accurate in determining sodium and potassium excretion as well as reflecting intake. In addition, the current studies do not often look specifically at the interaction effect of sodium and potassium consumption on cardiovascular disease. The proposed study was unique because it considered both the effects of sodium and potassium intake on individual cardiovascular health over a three-day period. This was accomplished by requiring the participants to keep a dietary record for three days and collecting urine for 24 hours at the end of the three days. The morning following the urine collection, endothelial function of the participants was assessed. Further, subjects were stratified based on their dietary intake of sodium and potassium. Those
with a high sodium, low potassium intake were grouped together while subjects consuming low sodium, high potassium intakes formed another group. It was hypothesized that the individuals with a higher sodium/potassium excretion ratio would be associated with a lower incidence of cardiovascular risk, as determined by measures of endothelial function.

OBJECTIVE: To examine the effects of habitual sodium and potassium intake on endothelial-dependent dilation in young healthy adults.

HYPOTHESIS: Habitual intake of a high sodium, low potassium diet would result in a greater sodium/potassium excretion ratio and inferior vasodilation as compared to those subjects consuming a low sodium, high potassium intake resulting in a lower sodium/potassium excretion ratio.
CHAPTER 4

METHODS

Thirty-two subjects were recruited for participation in this study. The Human Subjects Review Board at the University of Delaware (UD) approved all study procedures prior to data collection. All visits took place at the Vascular Physiology Research Lab at the University of Delaware, in Newark, Delaware. During the screening, subjects received a full verbal and written explanation of all procedures. Subjects signed an informed consent document approved by the Human Subjects Review Board at the University of Delaware (Appendix C). Participants were selected on a volunteer basis.

Participants

Both male and female subjects were recruited from the University of Delaware and surrounding area using flyers and the UD classifieds. Subjects were normotensive and pre-hypertensive (defined as BP 90-119/60-79 mmHg and 120-139/80-89 mmHg, respectively) and were apparently healthy, and between 18 and 31 years of age.

Exclusion Criteria

Subjects were excluded from participation in this study if they had a BP greater than 140/90 mmHg, were using cardiovascular and/or hypertensive medication, or were obese (defined as a body mass index > 30 kg/m²).

Screening Visit

The screening involved all of the following: completion of a medical history questionnaire (Appendix F); resting blood pressure (GE Medical Systems, Dinamap
Dash 2000, Milwaukee, WI); resting 12-lead electrocardiogram (Schiller AT-10, Electra-Med, Flint, MI); and height and weight measurement for the calculation of body mass index (Healthometer Scale, Continental Scale, Bridgeview, IL).

**General Protocol**

Completion of the study included two visits to the laboratory at the Vascular Physiology Lab (223 McDowell Hall; Newark, DE) or Human Performance Lab (541 South College Avenue; Newark, DE). The second visit took place on the last day of diet record recording and included blood work, and vascular function testing (see Figure 1 for timeline).

**Diet Record Instruction**

Subjects were instructed on how to keep a three-day food record, which was completed at home (Appendix A). On each recorded day, subjects began the food record on waking and kept track of all food and beverages consumed throughout the course of the day. Subjects were instructed to eat as they normally would, and to include two weekdays and one weekend day in their record. The instructions included helpful hints for keeping the most accurate log possible. Subjects found a detailed description of how to estimate portion sizes for fruits and vegetables, meats, nuts and other proteins, dairy, bread and grains, fats, and desserts. These descriptions were included in an effort to accurately monitor subjects’ food intake.

During their second visit to the HPL, the researchers had the opportunity to evaluate the diet record with the subject and correct for any error in estimation techniques. The subject’s second visit to the VPL immediately followed the final day
of the diet record. Each food record was analyzed for total energy, fat, protein, and carbohydrate consumption, as well as various vitamins and minerals using Nutrition Data System for Research (NDSR; Minneapolis, MN).

**Urine Collection**

Subjects were instructed to collect their urine for 24 hours in the provided container (Appendix B). Subjects began the urine collection approximately 24-hours before Visit 2 to the VPL. Subjects first emptied their bladder the morning of the urine collection, and then collected for a total of 24 hours; subjects were instructed to record time of their first void and time of their final void so that researchers could adjust for true urine time.

**Actical Physical Activity Assessment**

Physical activity of each subject was tracked by use of an Actical device, which is a physical activity monitoring system. Subjects wore the Actical (Respironics, Phillips Electronics) on their right hipbone on days coinciding with the three-day diet record (Appendix D). A velcro belt was provided for the subject to attach the Actical to the body. Subjects were instructed to start using the device upon waking on a chosen day of the recall, including during organized and unorganized physical activity. Activity levels were assessed using the Actical Software (Version 2.12) and ActiReader (Respironics, Phillips Electronics). A physical activity questionnaire (Appendix E) was administered to each subject on the second lab visit in case of malfunction with the Actical device.
Vascular Function Testing

Vascular function testing of the subjects was completed on the second visit to the VPL. The vascular function testing included central pulse wave analysis (PWA), carotid-femoral pulse wave velocity (PWV), and flow-mediated dilation (FMD) of the brachial artery of the non-dominant arm.

Subjects were asked not to eat for 4 hours, consume alcohol or caffeine for 12 hours, and exercise for 24 hours prior to this visit. During this visit, the subject had a 3 lead EKG applied to monitor their heart rate (HR) throughout the visit. First, subjects rested for ten minutes and a resting BP was taken. Next, PWA was performed (description of this method will be discussed later), followed by pressure recordings of the artery in the neck and groin, otherwise referred to as PWV. A non-stretchable cloth tape measure was used to measure the distance between the points on the neck and groin where these measurements were made so that velocity could be calculated for the PWV test. Following PWA and PWV, FMD of the brachial artery was performed.

Flow Mediated Dilation of the Brachial Artery

Measurement of endothelium-dependent flow mediated dilation (FMD) of the brachial artery was performed using a high-resolution ultrasound (Titan, Sonosite Inc.) equipped with an ECG. 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 to image the brachial artery longitudinally. A 4 MHz continuous wave Doppler probe (Multigon 500P) was positioned just distal to the ultrasound transducer. First, images and blood velocity were obtained for the
baseline measurement of end diastolic diameter and shear rate. Next, a cuff located below the elbow was inflated to 200 mmHg for five minutes. Fifteen seconds prior to and two minutes after release of the cuff, brachial artery images and blood velocity were recorded during the two minutes of reactive hyperemia for determination of peak diameter change\(^\text{50,51}\).

FMD was expressed as a percent increase in diameter from baseline (change in diameter divided by baseline diameter). LabView 8.6 was used to analyze brachial artery images using a known method with the addition of a customized edge-detection software program\(^\text{52}\). Video images were captured in LabView 10.0 using National Instruments IMAQ-PCI-1411 image acquisition board at a rate of 30 frames \(\cdot\) s\(^{-1}\). ECG and Doppler data were recorded simultaneously at 1000 Hz using a National Instruments PCI-6221 data acquisition board. Image size was converted to pixels to allow for determination of the diameter of the artery by utilizing reference marks placed on each image frame by the ultrasound. A vessel region of interest was then selected on a stable portion of the artery for determination of vessel diameter. The grayscale image was converted to binary and columns of pixels in the region of interest were analyzed to determine diameter. Within this image, 200-400 pixel columns were analyzed to determine diameter. Doppler data were analyzed in three-second time bins and shear rate was calculated using binned brachial diameters. Custom software determined baseline and peak diameter based upon changes in vessel diameter recorded from the ultrasound image. The software then calculated FMD.
**Pulse Wave Analysis**

To determine pressure waves for pulse wave analysis, a pencil-type force probe, or tonometer (Millar Instruments, Houston, TX) was placed over the radial artery. The tonometer was connected to the SphygmoCor Px system (AtCor Medical, Sydney, Australia) and the radial wave recorded. Based on this radial wave, a central pressure wave was non-invasively calculated via a transfer function. The best three radial wave recordings (highest reproducibility and operator indices) were averaged.

**Pulse Wave Velocity**

Carotid-femoral PWV was measured using tonometry to record both carotid artery and femoral artery waveforms simultaneously while the subject was at rest in a supine position. External distances were measured proximally from the carotid measurement site to the sternal notch, and distally from the sternal notch to the femoral measurement site via the navel. Carotid-femoral PWV was calculated by dividing the measured aortic distance (distal – proximal) by the average measured time delay between the initial upstrokes of ten consecutive corresponding carotid and femoral waveforms. Carotid-femoral PWV is a regional measurement of arterial stiffness.

**Blood Work**

A blood sample was taken after the subject arrived at the laboratory on the final day. The blood collected throughout the infusion was transferred to the appropriate vacutainer tube. For the collection of serum electrolytes and plasma osmolality, blood was spun for 15 minutes at 2,500 rpm in a centrifuge (Beckman...
Coulter, Allegra X-22R, Fullerton, CA). The serum potassium, sodium, and chloride were determined (Medica, EasyElectrolyte Analyzer, Bedford, MA). Plasma osmolality was measured (Advanced Instruments, Model 3D3 Osmometer, Norwood, MA). All measurements were in triplicate or quintuplicate and were performed after quality control standards were run. Whole blood was transferred to precalibrated capillary tubes and spun in triplicate on a Readacrit Centrifuge (Becton Dickinson, Clay Adams Brand, Parsippany, NJ) to determine hematocrit. Whole blood was also transferred to collecting slides for the analysis of hemoglobin (Hemocue, Hemocue Hb 201+ Analyzer, Lake Forest, CA).

Statistical Analysis

The statistical analysis for this project was performed using SPSS 19 (IBM, USA). The main variable being considered was the sodium to potassium excretion ratio. Pearson correlations were run to determine if an inverse relationship existed between the sodium/potassium excretion ratio and markers of vascular function. There was insufficient data to divide the subjects into two groups of those who have a low ratio, and those who have a high ratio. Data are presented as means and their standard error of measure (SEM). The statistical significance for all tests was set at p<0.05.

Human Subjects Approval

The approval for procedures to be followed to ensure confidentiality and practice was permitted by the by the Human Subjects Review Board of the University of Delaware (Appendix B). The informed consent forms were read, signed and dated by each participant prior to any involvement in the study. Files containing confidential
data were locked in a cabinet that only the researchers and committee members could access.
CHAPTER 5
RESULTS

Subject Characteristics

A total of 32 participants between the ages of 18 and 31 were recruited for this study with an average age of $23.8 \pm 0.64$ years. All subjects were non-hypertensive (defined as BP less than 140/90 mmHg) and the average SBP was $114 \pm 1.90$ mmHg and DBP was $63 \pm 1.54$. In addition, subjects were not overweight or obese as the average BMI was $23.53 \pm 0.52$ kg/m$^2$. The Actical data indicated that subjects were active with an average physical activity expenditure of $703.9 \pm 100.3$ kcal/day. Our subjects were not hypertensive, but several were in the pre-hypertensive range (n=9). The demographic data of the participants can be found in Table 1.

Diet Characteristics

Habitual sodium and potassium consumption varied greatly for the individuals who participated in this study. Sodium consumption ranged from 1962 mg/day to 9062 mg/day, with a mean intake of $3862.15 \pm 293.0$ mg/day. Potassium consumption ranged from 1227 mg/day to 4309 mg/day with a mean intake of $2922.2 \pm 148.2$ mg/day (Table 2). For a comparison of sodium and potassium consumption, refer to Figure 2. To ensure that participants were accurately recording their habitual dietary intake, sodium and potassium consumption levels were compared to sodium and potassium excretion values. Potassium consumed was significantly associated with potassium excreted (p<0.001; Figure 3). Sodium consumption was significantly associated with sodium excretion (p<0.001; Figure 4) as well. In addition, the
sodium/potassium excretion ratio was associated with both potassium consumption and sodium consumption (p=0.040 and p=0.024, respectively). For all subjects, as sodium consumption increased, the ratio of sodium/potassium excreted also increased (Figure 5). In contrast, as potassium consumption was increased, the sodium/potassium excretion ratio decreased, as expected (Figure 6).

When evaluating the relationship between BP and the sodium/potassium excretion ratio, an association between DBP and the ratio was seen for all subjects (p=0.098) suggesting that a high ratio correlated with a higher DBP (Figure 7). Further, sodium excretion alone significantly correlated with DBP (p = 0.032) and trended towards significance with SBP (p = 0.053).

The subjects’ total energy intake was also of interest. The range of calories consumed varied widely, from 1,337 kcals – 3,388 kcals/day, with a mean intake of 2,237 (+120 SEM). Males consumed more total energy and sodium than the females. Females consumed more potassium, calcium, and magnesium than men (Table 2).

The Institute of Medicine (IOM) provides a Dietary Reference Intake (DRI) for macro and micronutrients. The DRI for protein is 10-35% of total energy consumption, 45-65% of total energy for carbohydrates, and 20-35% of total energy for fat. For fat, carbohydrates, and protein, individuals ate within the DRIs (30.4% of total energy came from fat, 51.4% of total energy came from carbohydrates, and 16.3% of total energy came from protein). The DRI for calcium for the age group 18-30y/o is 800 mg/day (men) and 1,000 mg/day (women), magnesium is 330 mg/day (men) and 255 mg/day (women), potassium is 4,700 mg/day (all), sodium is <2,300
mg/day (all), and phosphorus is 580mg/day (all). Men and women were both consuming adequate amounts of calcium, magnesium, and phosphorus (976.5 mg/day and 1073.1 mg/day, 352.8mg/day and 389.3mg/day and 1477.8 and 1439.1 mg/day, respectably). Finally, as stated, all individuals were consuming too much sodium (3862.15 mg/day) and not enough potassium (3922.2 mg/day).

The IOM also provides DRIs for the different vitamins. Overall, our population was eating within the recommendations for most vitamins (Table 3). Both men and women consumed the DRI for Vitamin A, Vitamin K, Vitamin C, Thiamin, Riboflavin, Niacin, Vitamin B₆, folate, and Vitamin B₁₂, again suggesting that overall, our population kept a fairly healthy diet. Both men and women did not consume enough Vitamin D (men consumed 4.55 ± 0.65 mcg/day and women consumed 5.79 ± 0.77 mcg/day while the DRI is 15 mcg/day). Finally, men were eating enough Vitamin E, but on average, the women were falling just above the DRI for Vitamin E (15.38 ± 1.94 IU/s/day while the recommendation is 15 IU/s/day).

**Biochemical Parameters and Urinary Excretion Data**

Serum sodium and potassium and plasma osmolality were reported to ensure that participants were within a healthy range for these parameters (Table 4). Urinary excretion values were also reported and the sodium to potassium excretion ratio was calculated (24-hour sodium excretion/24-hour potassium excretion; Table 5).

**Vascular Outcomes**

There were a number of vascular measurements performed to determine endothelial function of study participants. These tests of vascular function included
central pulse wave analysis (PWA), carotid-femoral pulse wave velocity (PWV), and flow-mediated dilation (FMD) of the brachial artery of the non-dominant arm. After five minutes of occlusion, the average % change in brachial artery FMD for all subjects was $8.55 \pm 0.6 \%$ (n=27; Table 6). The mean was similar for men ($8.00 \pm 0.7 \%$) and women ($9.13 \pm 0.9 \%$) although the range for FMD varied greatly, with the greatest dilation at 15% and the smallest dilation at 4.68%. For FMD there was no association between FMD and sodium and potassium consumption nor with sodium and potassium excretion, however, data were missing on five individuals (Figure 11).

For all participants, there was a significant positive correlation between PWV and the potassium excretion, ($p=0.028, r=0.401$), however when looking at the relationship between PWV and the sodium/potassium excretion ratio, there was no statistical significance. It is worth noting that one subject had a very high augmentation index but as parameters to exclude data prior to starting the study were not established, the data have been included in the analysis (Figure 10). For all individuals, the average PWV was $4.86 \pm 0.27$ m/sec, with a range of $2.62 – 10.46$ m/sec (Table 6). There were no correlations between the augmentation index and sodium, potassium, or sodium/potassium excretion for all individuals (Figure 9). Average augmentation index was $0.0\% \pm 2.0$, with a range of $-16 – 27.7\%$ (Table 6).

The effect of sex on vascular function and sodium/potassium consumption as well as excretion was also examined. In males, augmentation index was positively correlated with the sodium/potassium excretion ratio ($p=0.031, r=0.557$; Figure 8) suggesting that a low sodium/high potassium diet is related to better vascular function.
in males. There was also a trend towards a positive correlation between sodium consumption and augmentation index (p=0.089, r =0.455) in men. In women, there was no association between sodium, potassium, or sodium/potassium excretion values and AI, PVW, or FMD (Table 6).
CHAPTER 6
DISCUSSION

The purpose of this study was to investigate the effect of habitual intake of sodium and potassium on endothelial-dependent dilation in a healthy, normotensive population. The hypothesis tested was that habitual intake of high sodium, low potassium diet would result in an inferior cutaneous vasodilation as compared to those subjects consuming a low sodium, high potassium intake. There are a few major findings from this study. First, as expected, individuals are consuming more sodium than recommended (2,300 mg/day) and less potassium daily than recommended (4,700 mg/day). Additionally, sodium consumption was correlated with sodium excretion (p<0.001), and potassium consumption was correlated with potassium excretion (p<0.001). The major vasculature measurement finding from this study was that in males, there is a positive relationship between AI (a measure of vascular function) and the sodium/potassium excretion ratio suggesting that a low sodium/high potassium diet is related to better vascular function.

Consumption Patterns

The 2010 Dietary Guidelines for Americans indicates that individuals are consuming sodium well above the recommendations (2,300 mg/day) and the findings of this study are consistent with this assertion. The 2010 Dietary Guidelines state that individuals 20-29 years old are consuming an estimated 4476 mg/day of sodium for men and an estimated 3107 mg/day for women. In this study, males consumed...
4422.4 ± 480.9 mg/day and women consumed 3301.9 ± 285.7 mg/day, both of which are consistent with the Dietary Guidelines data.

According to the 2010 Dietary Guidelines for Americans, individuals are also consuming far less potassium than the recommended levels (4,700 mg/day). Males 20-29 years old consume an average of 2,951 mg potassium/day, while women of the same age consume an average of 2,205 mg potassium/day. In this study, men consumed 2,779.4 ± 227.2 mg/day of potassium, while women consumed 3065 ± 190.7 mg/day. Both males and females fell well below the recommended intake for potassium however they are doing better than the national average.

The data presented in the Dietary Guidelines considers all individuals. Our study was unique in that it included only individuals with a BMI < 30 (kg/m²). Because of this selection criterion, the study population was fairly healthy and furthermore, was not overweight. In addition, individuals recruited for the study were between 18-40 years old. The mean age of individuals for the study was 24 years old, who are less likely to have a stiffening of their arteries.

**Excretion Data**

Sodium excretion data for our subjects ranged from 20.52– 397.8 mmol/24 hours, with a mean excretion of 161.5 mmol/24 hr ±14.8 (Table 5). O’Donnell et al. categorized urinary excretion into quintiles when describing the effect of sodium excretion on mortality rates. Based on their categorization, in this study, six individuals fell into the lowest quintile for excretion (<87 mmol/24 hr), fifteen fell into the second quintile (87-174 mmol/24 hr), eight fell into the third quintile (175-260
mmol/24 hr), one fell into the fourth quintile (260-348 mmol/24 h) and only two fell into the highest quintile (≥ 348 mmol/24 hr). That being said, approximately 2/3 (N=21) of our subject population fell into the low/very-low category for sodium excretion. This study aimed to determine the effect of high habitual sodium intake, but the individuals studied were not consuming high enough amounts of sodium even though they consumed more than the recommended amount compared to the literature. An additional explanation for the findings as they relate to sodium excretion values is that the subjects had excretion values that were very tightly clumped together. Differences in the vasculature may not be readily apparent with this group because of these similarities.

In terms of potassium excretion, the subject’s excretion ranged from 21.4 to 131.0 mmol/24 hr with a mean excretion of 66.9 ± 4.5 mmol/24 hour. Of these individuals, twenty-six of our subjects fell within 1SD of the mean, while 6 were more than 1SD above the average consumption. All but two subjects fell within 2SD of the mean. O’Donnell et al.33 evaluated potassium excretion and the risk of cardiovascular events. Again, quintiles were used to describe the effect of potassium excretion on mortality. Based on the categorization presented by O’Donnell et al., for our study, three individuals fell into the lowest excretion quintile (<38.4 mmol/24 hr), six fell into the second quintile (38.5 – 50.9 mmol/24 hr), seven fell into the third quintile (51.0 – 63.7 mmol/24 hr), seven fell into the fourth quintile (63.8 – 76.7 mmol/24 hr), and nine fell into the highest potassium excretion quintile (>76.8 mmol/24hr) suggesting that our subjects on average were consuming more potassium. O’Donnell
found no significant association between potassium excretion and CV mortality, MI, and CHF, so it is not surprising that the expected protective effects of potassium excretion on the vasculature were not seen. Therefore, looking at potassium excretion alone may not have any clinical significance.

Finally, for the sodium/potassium excretion ratio, the range was 0.37 – 9.51 mmol sodium/mmol potassium. The mean excretion value was $2.70 \pm 0.3$ mmol sodium/mmol potassium, with twenty-six individuals falling within 1SD of the mean. All but one individual fell within 2SD of the mean. Again, the excretion ratio for participants was concentrated and very low, which may have masked the vascular effects that we were considering for this study. Therefore, a larger sample size may be needed to see significance.

In a cross-sectional study by Huggins et al., the relationship between the presence of hypertension and the sodium to potassium excretion ratio was evaluated. Participant’s age ranged from 27–75 years old, with a mean age of 64 and an average BMI of 28.2 $(m/kg^2)$. The excretion ratio was divided into quintiles, much like previous work. The quintiles for excretion ratio were defined as the following: Quintile 1 was $<1.31$, Quintile 2 was 1.31–1.67, Quintile 3 was 1.68 – 2.06, Quintile 4 was 2.06 – 2.61, and Quintile 5 was $>2.61$. In looking at our participants, six individuals fell into the lowest excretion quintile, none fell into Q2, eight fell into Q3, seven fell into Q4 and 11 fell into the highest quintile. Huggins et al. found that a higher sodium/potassium excretion ratio was associated with a high SBP and DBP, which is consistent with the findings of this study. In their model, 36% of individuals
in Q1, 46% of individuals in Q2, 36% of individuals in Q3, 48% of individuals in Q4 and 52% of individuals in Q5 were hypertensive, supporting the idea that a higher sodium/potassium excretion ratio is associated with hypertension. The same effects (despite our similar stratification of individuals across each quintile) were most likely not observed, because the population of interest was so much younger (mean age of 24 compared to a mean age of 64 in the study by Huggins et al.).

**Augmentation Index**

In our study, the augmentation index, which is a measure of vascular function, was lower for males with a lower sodium/potassium excretion ratio compared to those with a high sodium/potassium excretion ratio (p=0.031, r=0.557). AI is dependent on the elastic properties of the branching pattern of the arteries, the velocity of the reflected wave, and the distance to the reflected site. That being said, low AI indicates that an individual has more elasticity of the arteries. Our data indicate that as the sodium/potassium ratio is increased, there is less arterial elasticity for the males. This relationship between cardiovascular health and the sodium/potassium excretion ratio is support by literature for older individuals (55+ y/o).

**Flow Mediated Dilation:**

There was no correlation between FMD and the sodium/potassium ratio with this population. Jablonski et al. recently reported an association between a low dietary sodium intake and enhanced vascular endothelial function (measured with FMD) in healthy middle-aged and older adults (48-73 y/o). These researchers defined low dietary sodium intake as <100 mmol/day (or <2,300 mg/day), while normal intake
was 100-200 mmol/day (2,300-4,600 mg/day). Only six of our subjects consumed <2,300 mg/day of sodium, which may have made it more difficult to see the effects of the diet on FMD. In those subjects that consumed less than 100 mmol/day of sodium their FMD was 8.68 ± 1.17% which is very close to the group average.

In a study done by Dickinson et al.\textsuperscript{37}, FMD was assessed in young, healthy individuals. Individuals were 37.4 years old with a mean BMI of 24.8 kg/m\textsuperscript{2} and normotensive BP. FMD was measured in the individuals before and after a low-salt meal and a high-salt meal. Both meals were similar in their nutrient content except for the salt content: the high-salt meal contained 1494 mg of sodium and the low-salt meal contained 130 mg of sodium. Individuals eating the low-salt meal had an average FMD of 8.35%, while those on the high-salt diet had a FMD of 7.61%. Although we cannot compare these results directly to the findings in this study (as we measured FMD after a four hour fast and we did not control their dietary intake), the investigators are confident that the mean FMD % change is consistent with the literature for healthy individuals.

In another study done by Dickinson et al.\textsuperscript{36}, researchers tested the effect of salt reduction on the FMD of the brachial artery. Subjects were older than our population (mean age 52.7 y/o), had a slightly higher BMI (31.6 kg/m\textsuperscript{2}), and were normotensive. Twenty-nine subjects were provided either a low-salt or usual-salt diet, where all dietary components were similar except for sodium content. Sodium content of the low-salt diet was 730.4 mg/day while the sodium content of the usual salt diet was 2048.4 mg/day. Researchers did collect information on dietary potassium, and it was
similar between groups (3186.5 mg/day for low-salt and 3024.2 mg/day for usual salt). The percent change in FMD was significantly greater in the low-salt diet group (4.89%) compared to the usual salt diet group (3.37%). PWV and AI did not differ significantly between each diet group. The mean FMD of the individuals in this trial was lower than our mean FMD, which may be due to the age difference of the individuals in each study or differences between technicians performing the measurement. Additionally, subjects in our study were consuming considerably more sodium than either diet group in this study. It is also possible that because our subjects are young and healthy, the changes in their vasculature in response to sodium intake may not be noticeable at such a young age.

**Pulse Wave Velocity:**

There was no correlation between the sodium/potassium excretion ratio and PWV for this population. Average PWV for our subjects was 4.86 ± 0.27 m/sec. Todd et al.\(^{55}\) conducted a study that examined the effects of dietary salt intake on vascular function in hypertensive, older individuals (mean age 51.8 y/o). PWV and PWA were the measures used to determine vascular function. Participants were put on one of three dietary interventions: intervention A was a diet containing a dietary sodium content of 1,380 mg/day, intervention B was 3,450 mg/day, and intervention C was 4,600 mg/day. Subjects were instructed to follow the intervention for 4 weeks. For PWV, the difference between intervention B and A was 0.39 m/sec and the difference between intervention C and A was 0.35 m/sec, suggesting that a low salt diet is associated with a lower PWV, which is indicative of better vascular health. Todd et al.
did not find significant differences between intervention B and C, which is consistent with our findings. Although individuals on intervention A displayed better vasculature as measured by PWA, interventions B and C were not different. Our subjects consumed an average of 3900 mg sodium/day, which is between interventions B and C. Therefore, it is not surprising differences in the vasculature as measured by PWV were not seen. Additionally, the fact that our subjects were young and healthy may have made the differences harder to detect.

*Limitations:* The study subjects were asked to independently collect a 24-hour urine in order to determine sodium/potassium excretion ratio. Some subjects were very accurate in recording the actual time of first and final void (so that researchers could determine the total excretion collection time) while others were not. This inaccuracy in reporting could have led to inaccurate excretion data. In an effort to control for this, excretion data were compared to intake data, and the relationships were significantly similar. Still, having actual excretion times for all participants would have been ideal in order to express sodium and potassium excretion. If time was not available, it was assumed that the subject collected for a full 24 hours. Subjects were asked to keep a three-day food record during the study. Individuals were instructed on the best methods to estimate portion sizes (Appendix A) and were given examples of portion sizes. However, dietary intake was not observed and there is the possibility that the records are missing some dietary intake.

The sample size for this cross-sectional study was small, which may have made it more difficult for researchers to find significance, particularly with the
measurement of FMD. For this reason, it would be beneficial to continue recruiting subjects to reach numbers closer to 40-50, and numbers that were trending at 30 may present themselves as significant. Finally, the investigators did not control for the menstrual cycle in our study. Research has shown that FMD values can vary over the menstrual cycle by 5-7\%{56}.

**Strengths:** To our recognition, this study is the first of its kind to consider the relationship between habitual sodium and potassium intake and vascular function in young, healthy individuals. In order to determine the sodium and potassium excretion, a 24-hr urine collection was assessed, which is the gold standard for determining excretion values. This value was then compared to reported intake in order to determine accuracy of reporting. Additionally, subject dietary records were reviewed during their second visit to the lab, to clarify any missing information.

**Perspectives:** There are a number of studies that indicate that middle age and older individuals (both with and without hypertension and other CV related disease states) benefit from a low sodium/high potassium diet. Our study was unique in that it assessed habitual intake of sodium and potassium in individuals aged 18-40 years (with a mean age of 24). Significance with many of our variables was not found, which may have occurred due to the following reasons. One, because our population of interest was young, they may not be experiencing the ill effects of a high salt diet on the vasculature. If an older population were studied, this effect may have been more evident. Additionally, overall sodium consumption was high, with only six individuals consuming below the recommended levels of 2,300 mg/day. Most of the
existing literature focuses on a reduced sodium diet, which is not descriptive of the habitual diet of our subjects. Finally, our subjects were all fairly healthy, with an average BMI of 23.8 kg/m², indicating that they do not have obesity as a comorbidity. For these reasons, it would be interesting to look at habitual sodium and potassium intakes and excretion in a normotensive, healthy population that is a bit older (40-60 years) but still fits within our inclusion and exclusion criteria.

Conclusions: The present findings of this study provide support that there is a relationship between sodium/potassium excretion ratio and augmentation index in males. However, further research needs to be done in order to determine whether there is a strong relationship between this excretion ratio and FMD, PWA, and PWV in both sexes.
Table 1: Demographic Data

<table>
<thead>
<tr>
<th>Demographic Data</th>
<th>Mean ± SEM</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>23.84 ± 0.6</td>
</tr>
<tr>
<td>Gender</td>
<td>16M, 16F</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172.44 ± 9.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.80 ± 2.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.53 ± 0.5</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>113.90 ± 1.9</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>62.98 ± 1.5</td>
</tr>
<tr>
<td>Actical Expenditure (kcal)</td>
<td>703.9 ± 100.4</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>14.21 ± 0.3</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40.86 ± 0.7</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>57.8</td>
</tr>
</tbody>
</table>
Table 2: Dietary Intake

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>All Subjects (N=32)</th>
<th>Males (N=16)</th>
<th>Females (N=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy (kcalories)</td>
<td>2237 ± 120</td>
<td>2526 ± 186.9</td>
<td>1949 ± 116.2</td>
</tr>
<tr>
<td>FAT (g), % from FAT</td>
<td>79.0 ± 5.6 (30.4%)</td>
<td>92.3 ± 7.6 (32.0%)</td>
<td>65.6 ± 7.2 (28.8%)</td>
</tr>
<tr>
<td>CHO (g), % from CHO</td>
<td>291.1 ± 16.4 (51.4%)</td>
<td>317.0 ± 27.3 (50.0%)</td>
<td>265.3 ± 16.6 (53.4%)</td>
</tr>
<tr>
<td>PTN (g), % from PTN</td>
<td>92.26 ± 4.7 (16.3%)</td>
<td>100.5 ± 7.2 (15.4%)</td>
<td>84.0 ± 5.5 (17.2%)</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>3862.15 ± 293.0</td>
<td>4422.4 ± 480.9</td>
<td>3301.9 ± 286.0</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>2922.2 ± 148.2</td>
<td>2779.4 ± 227.2</td>
<td>3065 ± 190.7</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1024.8 ± 61.7</td>
<td>976.5 ± 96.6</td>
<td>1073.1 ± 78.1</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>371.0 ± 24.7</td>
<td>352.8 ± 40.9</td>
<td>389.3 ± 28.2</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>1458.4 ± 61.2</td>
<td>1477.8 ± 101.9</td>
<td>1439.1 ± 71.0</td>
</tr>
<tr>
<td>Caffeine (mg)</td>
<td>103.5 ± 17.9</td>
<td>67.1 ± 17.7</td>
<td>139.9 ± 28.84</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>7.0 ± 2.8</td>
<td>11.9 ± 5.2</td>
<td>2.23 ± 1.31</td>
</tr>
</tbody>
</table>

All data reported as mean ± SEM.
Table 3: Vitamin Intake

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>All Subjects (N=32)</th>
<th>Males (N=16)</th>
<th>Females (N=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A (mcg)</td>
<td>1428 ± 163.24</td>
<td>1272.38 ± 217.9</td>
<td>1583.62 ± 243.78</td>
</tr>
<tr>
<td>Vitamin D (mcg)</td>
<td>5.17 ± 0.51</td>
<td>4.55 ± 0.65</td>
<td>5.79 ± 0.77</td>
</tr>
<tr>
<td>Vitamin E (IU)</td>
<td>17.66 ± 1.78</td>
<td>19.94 ± 2.9</td>
<td>15.38 ± 1.94</td>
</tr>
<tr>
<td>Vitamin K (mcg)</td>
<td>161.17 ± 26.48</td>
<td>114.6 ± 20.44</td>
<td>207.73 ± 46.81</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>123.53 ± 13.93</td>
<td>112.30 ± 20.74</td>
<td>134.75 ± 18.86</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>2.0 ± 0.12</td>
<td>2.26 ± 0.20</td>
<td>1.73 ± 0.11</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>2.42 ± 0.15</td>
<td>2.53 ± 0.21</td>
<td>2.30 ± 0.21</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>29.04 ± 1.74</td>
<td>31.54 ± 2.54</td>
<td>26.54 ± 2.29</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>3.00 ± 0.70</td>
<td>2.33 ± 0.20</td>
<td>3.67 ± 1.40</td>
</tr>
<tr>
<td>Folate (mcg)</td>
<td>571 ± 46.54</td>
<td>628.94 ± 79.90</td>
<td>513.06 ± 46.03</td>
</tr>
<tr>
<td>Vitamin B12 (mcg)</td>
<td>4.97 ± 0.47</td>
<td>4.90 ± 0.61</td>
<td>5.04 ± 0.74</td>
</tr>
</tbody>
</table>

All data reported as mean ± SEM.
Table 4: Biochemical Parameters

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Mean ± SEM (N=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Sodium (mmol/L)</td>
<td>138.0 ± 0.28</td>
</tr>
<tr>
<td>Serum Potassium (mmol/L)</td>
<td>4.24 ± 0.06</td>
</tr>
<tr>
<td>Plasma Osmolality (mOsm)</td>
<td>285.6 ± 0.50</td>
</tr>
</tbody>
</table>
Table 5: Urine Excretion Data

<table>
<thead>
<tr>
<th>Urinary Markers</th>
<th>Mean ± SEM (N=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine Osmolality</td>
<td>557.2 ± 41.9</td>
</tr>
<tr>
<td>24-Hour Sodium Excretion (mmol/24 hr)</td>
<td>161.1 ± 14.8</td>
</tr>
<tr>
<td>24-Hour Potassium Excretion (mmol/24 hr)</td>
<td>66.9 ± 4.5</td>
</tr>
<tr>
<td>24-Hour Sodium/Potassium Excretion</td>
<td>2.70 ± 0.3</td>
</tr>
</tbody>
</table>
Table 6: Measures of Vascular Function

<table>
<thead>
<tr>
<th>Vascular Measurement</th>
<th>All Subjects (N=32)</th>
<th>Males (N=16)</th>
<th>Females (N=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Males (N=16)</td>
<td>Females (N=16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FMD (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td>Brachial Artery</td>
<td>8.55 ± 0.6</td>
<td>8.00 ± 0.7</td>
<td>9.13 ± 0.9</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>(N=27)</td>
<td>(N=14)</td>
<td>(N=13)</td>
</tr>
<tr>
<td></td>
<td>4.68 – 15</td>
<td>4.68 – 12.44</td>
<td>5.47 – 15</td>
</tr>
<tr>
<td>Augmentation Index</td>
<td>0.0 ± 2.0</td>
<td>-2.42 ± 2.4</td>
<td>2.57 ± 3.4</td>
</tr>
<tr>
<td>Index (%)</td>
<td>(N=29)</td>
<td>(N=15)</td>
<td>(N=14)</td>
</tr>
<tr>
<td></td>
<td>-16 – 27.7</td>
<td>-15.7 – 20.3</td>
<td>-16.0 – 27.7</td>
</tr>
<tr>
<td>Pulse Wave Velocity</td>
<td>4.86 ± 0.27</td>
<td>5.29 ± 0.41</td>
<td>4.07 ± 0.31</td>
</tr>
<tr>
<td>(m/sec)</td>
<td>(N=30)</td>
<td>(N=16)</td>
<td>(N=14)</td>
</tr>
<tr>
<td></td>
<td>2.62 – 10.46</td>
<td>3.79 – 10.46</td>
<td>2.62 – 6.32</td>
</tr>
</tbody>
</table>

All data reported as mean ± SEM.
FIGURES
Figure 1: Participant Timeline for Lab visits
Figure 2: Sodium and Potassium intake (mg/day) for all subjects
Figure 3: Association between Potassium consumed (mg/day) and Potassium excreted (mmol/24 hr) for all subjects
Figure 4: Association between Sodium consumed (mg/day) and Sodium excreted (mmol/24 hr) for all subjects
Figure 5: Association between Sodium consumed (mg/day) and Sodium/Potassium excretion ratio for all subjects
Figure 6: Association between Potassium consumed (mg/day) and Sodium/Potassium excretion ratio for all subjects
Figure 7: Association between DBP (mmHg) and Sodium/Potassium excretion ratio for all subjects
Figure 8: Association between Augmentation Index (%) and Sodium/Potassium excretion ratio for males
Figure 9: Association between Augmentation Index (%) and Sodium/Potassium excretion ratio in all subjects
Figure 10: Association between Pulse Wave Velocity (m/sec) and Sodium/Potassium excretion ratio in all subjects
Figure 11: Association between percent Change in Brachial Artery Flow Mediated Dilation and Sodium/Potassium excretion ratio in all subjects
REFERENCES

16. Ogunniyi MO, Croft JB, Greenland KJ, Giles WH, Mensah GA. Racial/ethnic differences in microalbuminuria among adults with prehypertension and
31. Hebert PR, Bolt RJ, Borhani NO, et al. Design of a multicenter trial to evaluate long-term life-style intervention in adults with high-normal blood pressure.


Appendix A

INSTRUCTIONS FOR KEEPING YOUR THREE-DAY FOOD 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: _______________

<table>
<thead>
<tr>
<th>Meal/Time of Day</th>
<th>Food/Drink (specify brand or restaurant name)</th>
<th>Amount</th>
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FOOD INTAKE LOG

Day of the Week: ______________

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FOOD INTAKE LOG

Day of the Week: ______________

<table>
<thead>
<tr>
<th>Meal/Time of Day</th>
<th>Food/Drink (specify brand or restaurant name)</th>
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Appendix B

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, Taylor Schellhardt, BS, 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 40 participants. We are recruiting both men and women aged 18-40 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. 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 1½ 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.

The following will occur:
• Resting heart rate, blood pressure, height, and weight will then be measured again.
• A 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.

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 no 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)-831-2137.

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
Subject’s Signature

Date:_____________________

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 C

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)

  o Shannon Lennon-Edwards, PhD 302-382-4291 (cell); 302-831-2798 (office)
  o Bill Farquhar, PhD 302-397-1591 (cell)

*** Questions about Urine Collection?? ***

call TAYLOR: 770-401-8411
Appendix D

INSTRUCTIONS FOR ACTICAL® PHYSICAL ACTIVITY MONITOR

- You will be asked to wear the Actical for 24 consecutive hours on each of the three days of your dietary recall. 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. At the end of each 24 hour recording period, press **STAR** again.

- 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**

- Shannon Lennon-Edwards, PhD 302-382-4291 (cell); 302-831-2798 (office)
- Taylor Schellhardt 770-401-8411 (cell)
Appendix E

PHYSICAL ACTIVITY QUESTIONNAIRE

The Seven-Day Recall

<table>
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**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:

<table>
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<tr>
<th>Rounding: 10-22 min = .25</th>
<th>1.08-1.22</th>
<th>2.23-37 min = .50</th>
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<td>An asterisk (*) denotes a work-related activity.</td>
<td>38-52 min = .75</td>
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<td>A squiggly line through a column (day) denotes a weekend day.</td>
<td>53-107 min = 1.0</td>
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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 F

MEDICAL HISTORY AND GENERAL INFORMATION FORM
Microvascular Function and Sodium Chloride

Name: _______________________________  Date: _______________
Address:

_____________________________________________________________________
City: ___________________  State: _______  ZIP: _______________
Phone: ____________________  Age: _______________

Emergency Contact: ____________________  Phone: ____________________
Do you have a family physician?  ____ YES  ____ NO
If yes, Physician’s Name:

_____________________________________________________________________
Physician’s Address:

_____________________________________________________________________
City: ___________________  State: _______  ZIP: _______________
Do you have a nephrologist (kidney doctor)?  ____ YES  ____ NO
If yes, Physician’s Name:

_____________________________________________________________________
Physician’s Address:

City: ___________________  State: _______  ZIP: _______________

Do you have or have you had:
Diabetes Mellitus:  ____ YES  ____ NO
Kidney or bladder problems:  ____ YES  ____ NO
Anemia:  ____ YES  ____ NO
Asthma:  ____ YES  ____ NO
Other lung problems:  ____ YES  ____ NO
High blood pressure:     ____ YES ____ NO
Coronary heart disease:   ____ YES ____ NO
Heart attack:             ____ YES ____ NO
Heart failure:            ____ YES ____ NO
Stroke:                  ____ YES ____ NO
Irregular heart rhythm:   ____ YES ____ NO
Other heart problems     ____ YES ____ NO (specify)
Cancer:                  ____ YES ____ NO
Gout:                    ____ YES ____ NO
Blood disorders:         ____ YES ____ NO
Any other significant
or chronic problems:     ____ YES ____ NO

Do you ever have pain in your chest? ____ YES ____ NO
Do you ever have pain in your chest while exercising? ____ YES ____ NO
Do you ever have any arm or jaw discomfort, nausea, or vomiting associated with
cardiac symptoms? ____ YES ____ NO
Have you ever smoked:
    Cigarettes: ____ YES ____ NO
    Pipe:       ____ YES ____ NO
    Cigar:      ____ YES ____ NO
If yes, how many per day? ___________________
Do you currently smoke? ____ YES ____ NO
How long have you smoked? ___________________
Do you drink caffeinated beverages? ____ YES ____ NO
Are you taking any medications or supplements? ____ YES ____ NO
If yes, please list (please include supplements, vitamins, and antioxidants):

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<tr>
<th>Name</th>
<th>Dose</th>
<th>Number of times/day</th>
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Appendix G
IRB Human Subjects Approval

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.
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.
DATE: July 25, 2011

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

STUDY TITLE: [180191-3] The Relationship between Habitual Salt and Potassium Intake on Vascular Function

SUBMISSION TYPE: Continuing Review/Progress Report

ACTION: APPROVED
APPROVAL DATE: July 25, 2011
EXPIRATION DATE: July 25, 2012
REVIEW TYPE: Expedited Review

REVIEW CATEGORY: Expedited review category # 9

Thank you for your submission of Continuing Review/Progress Report 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.

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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 jIberg@udel.edu. Please include your study title and reference number in all correspondence with this office.