THE ROLE OF HABITUAL POTASSIUM AND SODIUM INTAKE ON VASCULAR FUNCTION IN HEALTHY ADULTS

by

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ABSTRACT

Cardiovascular disease (CVD) is the number one killer in U.S. Endothelial dysfunction is considered an underlying non-traditional risk factor for CVD. High sodium intake has been linked with incidents of hypertension and CVD while high potassium intake has been linked with lower incidence. Independent of blood pressure (BP), a high sodium intake has shown to cause endothelial dysfunction however this association has been predominantly under controlled conditions. Assessment of vascular function on a habitual diet remains to be investigated. Therefore, the aim of this study was to determine the relationship between habitual sodium and potassium intake on vascular function in healthy adults. We hypothesized that high potassium/low sodium diets would result in greater endothelial-dependent dilation as determined by brachial artery flow mediated dilation (FMD) and nitric oxide (NO)-mediated cutaneous vasodilation in response to local heating in healthy adults and second that low potassium/high sodium diets would have an improvement in cutaneous vasodilation in response to infusion of antioxidants compared to subjects with a higher potassium/lower sodium diet. Nine healthy subjects aged 28.8 ± 4 years completed the assessment of vascular function that included brachial artery FMD and cutaneous microvascular function while consuming their habitual diet. Subjects

underwent 24-hr BP monitoring and collected their urine for 24 hours for analysis of sodium and potassium concentrations. Three-day diet records were used to assess habitual intake and a 24-hour recall was given the day of the study visit to compare to urinary sodium excretion. The 24-hour recall of sodium and potassium did not significantly correlate with 24-hour urinary excretion. Twenty-four BP monitoring revealed that all subjects were normotensive. The average brachial artery FMD was 6.24 ± 1.26%. However, there was no correlation between habitual sodium, potassium, or the ratio of sodium to potassium with FMD. In regards to microvascular function, there were no baseline or NO-mediated plateau differences between sites. However, there was a relationship between the NO plateau for the ascorbic acid site with sodium intake ($R = 0.7650$; $P < 0.05$) suggesting that those individuals with a higher sodium intake were more responsive to ascorbic acid. However, no other relationship between sodium and potassium intake with vascular function was found. In conclusion, this study found that administration of ascorbic acid improved the NO-mediated plateau in those with a higher sodium intake suggesting a role for oxidative stress. However, no other relationship between sodium and potassium intake with vascular function was found.

Chapter 1

INTRODUCTION AND BACKGROUND

1.1 Cardiovascular Disease and Risk Factors

Cardiovascular disease is the number one killer in the US and accounts for 1 in every 3 deaths. Over $312.6 billion in direct and indirect costs are attributed to CVD every year (Mozaffarian et al., 2016). Cardiovascular disease refers to a variety of diseases including, but not limited to coronary artery disease, heart failure, arrhythmias, heart valve disease, peripheral artery disease, high blood pressure, stroke, myocardial infarction, and congenital heart disease (Mozaffarian et al., 2016). Major risk factors for CVD include age, sex, race, family history, smoking, unhealthy diet, blood pressure, cholesterol, diabetes, obesity, physical inactivity, stress, and poor hygiene (Mozaffarian et al., 2016). Risk increases with age, and men are at a greater risk than pre-menopausal women. Some of these risks are avoidable, and CVD risk can be decreased by not smoking, maintaining a healthy weight, living an active lifestyle, and eating a healthy diet low in sodium, fats, and cholesterol (Brown et al., 2001).

1.2 Endothelium

The endothelium is a single layer of cells lining the blood vessels. The endothelium is responsible for maintaining vascular tone, vascular homeostasis, and modulating leukocyte and platelet adhesion (Brown et al., 2001; Hadi et al., 2005). The endothelium releases several vasoactive substances in response to stimuli such as sheer stress on the vessel from increased flow (Brown et al., 2001; Hadi et al., 2005.) The vasodilators include

nitric oxide (NO), endothelium derived hyperpolarizing factor (EDHF), and prostacyclin (Cooke et al., 2000). The vasoconstrictors include thromboxane A2, prostaglandin H2, and endothelin 1 (Shimokawa et al., 1999). The homeostatic balance between the constrictors and dilators determines tone in the vasculature.

Endothelial cells are also necessary for cell adhesion and thrombosis, as they express adhesion molecules such as P-selectin, E-selectin, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and cytokines, which have important roles in platelet adhesion during thrombosis, a beginning step of atherosclerosis (Springer et al., 1999). In times of inflammatory stress, elevated levels of oxidative stress, and high cytokine expression, the endothelium becomes activated and up regulates the expression of these molecules. This activation can lead to atherosclerosis when monocytes in the blood bind to the adhesion molecules, which the inflamed endothelial cells overexpress, and enter the endothelium where they become macrophages. The macrophages consume oxidized LDL and become foam cells, which form the plaques leading to atherosclerosis. Most CVD is related to atherosclerosis, which is the build up of plaque in the arteries that may cause obstruction of blood flow leading to stroke or heart attack (Ross et al., 1999). One reason hypertension is a risk factor for a myocardial infarction is because increased pressure can cause plaque to break off and cause a thrombosis, which can block blood flow to the heart, causing a myocardial infarction (Harbaoui et al., 2015).

1.2.1 Nitric Oxide

Nitric oxide is an important vasodilator that is formed in the endothelial cells and is responsible for assisting in blood vessel dilation (Hadi et al., 2005). Three forms of NO exist including endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), and inducible nitric oxide synthase (iNOS). eNOS is the most important of the three in regards to NO in the vasculature (Berka et al. 2014). Nitric oxide is formed in the endothelium by the conversion of L-arginine to L-citrulline through eNOS (Holowatz et al., 2011; Vasquez-Vivar et al., 1998). Cofactors required for eNOS activation include nicotinamide adenine dinucleotide (NADPH), flavin adenine mono- and di- nucleotides (FAD/FMN) and tetrahydrobiopterin (BH4). Once NO is formed, it diffuses into the smooth muscle cells and causes cyclic GMP formation, which assists in muscle relaxation/vasodilation (Shimokawa et al., 1999). Nitric oxide production is increased when blood flow decreases, in a negative feedback loop due to an influx of calcium and calcium release from the cell (Fleming et al., 1997). In addition to vasodilation, NO inhibits several vasoconstrictors (Cooke et al., 2000). Further, the presence of NO can inhibit platelet aggregation, LDL oxidation, monocyte adhesion, smooth muscle proliferation and migration, cytokine synthesis, angiotensin II, and endothelin I (Cooke et al., 2000).

1.2.2 Endothelial Dysfunction

The mechanisms involved in the development of atherosclerosis provide evidence that alterations in the endothelium, called endothelial dysfunction, is a precursor to CVD. Endothelial dysfunction is functionally

defined as an impairment of endothelium-dependent vasodilation and a state of endothelial activation characterized by inflammation and proliferation (Cooke et al., 2000; Hadi et al., 2005).

Risk factors for developing endothelial dysfunction are similar to those for developing CVD and include smoking, aging, family history, high cholesterol, hyperglycemia, and high blood pressure (Halcox et al., 2009). Endothelial dysfunction can also be seen independent of high blood pressure (DuPont et al., 2013; Greaney et al., 2012). Endothelial dysfunction has been shown to be a generally reversible factor in the pathway of CVD for many people (Hadi et al., 2005). Reducing risk factors for CVD and hence, endothelial dysfunction through lifestyle factors as well as antihypertensive therapy and ACE inhibitors has been shown to slow progression of disease (Hadi et al., 2005). Additionally, dietary factors such as omega-3 fatty acids, antioxidants, folic acid, and L-arginine have been shown to improve endothelial function (Cooke et al., 2000).

1.2.3 Oxidative stress

Reactive oxygen species (ROS) are molecules generated by injury and inflammation that can cause vascular dysfunction and disease (Harrison et al., 2007). Enzymes such as NAD(P)H oxidase, xanthine oxidase, mitochondrial electron transport enzymes, and nitric oxide synthase can transfer electrons from their outer shells to oxygen under certain conditions. A one-electron oxidation of oxygen results in superoxide ($O_2^{\bullet -}$), a common oxidant (Harrison et al., 2007). At low concentrations, ROS can act as signaling molecules participating in cell regulation and adaptation responses (Hadi et al., 2005). At

higher concentrations, ROS can cause injury and death (Hadi et al., 2005), with the endothelium being a primary target for ROS since it is permeable. ROS increase the permeability of the endothelium and promote leukocyte adhesion, which leads to atherosclerotic plaque build up (Harrison et al., 2007). Endothelial dysfunction may also lead to plaque rupture, which can cause a coronary event such as myocardial infarction or stroke (Hetizer et al., 2001). Disruption of the L-arginine NO pathway by ROS has been suggested as a mechanism responsible for the increased endothelial dysfunction seen in individuals with salt-sensitive hypertension (Bragulat et al., 2001). Low potassium has also been shown to increase incidence of hypertension (Whelton et al., 1997). Finally, Vitamin C, a nonspecific antioxidant has been shown to improve endothelial function suggesting a role for oxidative stress in endothelial dysfunction (Tadei et al., 1994).

Sources of free radicals that uncouple nitric oxide synthase include xanthine oxidase and NADPH oxidase (Harrison et al., 2007). In particular, mitochondrial sources are the main producers of ROS, specifically $O_2^{\bullet-}$ (Harrison et al., 2007). Oxidative stress levels and in particular $O_2^{\bullet-}$, is increased during hypertension, which has been linked with salt intake (Harrison et al., 2007; Hummel et al., 2012). Under normal conditions, eNOS with the cofactor BH4, forms NO. However, oxidative stress can uncouple BH4 resulting in the oxidation of NO by $O_2^{\bullet-}$ that produces peroxynitrite, a potent ROS. The result is a loss of NO and hence, an increase in vasoconstriction (Harrison et al., 2007).

1.3 Sodium

1.3.1 Role of Sodium in the Body

Sodium is an essential mineral that the human body needs to maintain electrolyte balance, muscle and nerve function, as well as regulate blood pressure and volume (IOM, 2005; DGA, 2015). Sodium is found in small amounts in almost all foods, but is high in processed, canned, and commercially prepared foods. Too much sodium in the diet has been linked to increased blood pressure and risk of CVD (Mozzafarian et al., 2016; Tuomilehto et al., 2001).

The adequate intake (AI) for sodium is 1,500mg per day, and it's upper limit (UL) is 2,300mg per day for male and female adults. (IOM, 2005; DGA, 2015). In contrast, the amount needed to maintain homeostasis in the body is less than 500mg per day (Bernstein et al., 2010). It is recommended that all adults, but especially those who are 51 years and older, are African American, have chronic kidney disease, diabetes, or high blood pressure should consume 1,500 mg per day or less. The average daily amount of sodium consumed by individuals in the U.S. (includes those age 2 and older) is 3,440mg (DGA, 2015), which is 1.5 times higher than the recommendation. According to the American Heart Association, 32.6% of adults in the United States between 2009-2012 had diagnosed hypertension (Mozaffarian et al., 2016). Consuming excess sodium has been proven to be a risk factor for CVD mortality and high blood pressure (Mozaffarian et al., 2016).

1.3.2 Sodium and Hypertension

Sodium is crucial to normal body function, but can have negative effects when consumed in excess or when not enough is consumed (Meneton et al., 2005). Diets high in sodium content result in fluid retention in the body. This extra fluid makes the heart work harder, resulting in increased blood pressure (Titze et al., 2014). The body maintains homeostasis by keeping sodium in the plasma and the interstitium equally distributed, but when sodium intake increases, osmolality causes increased water retention in the vasculature, therefore increasing volume and pressure. The opposite occurs when sodium intake is below the requirements (Titze et al., 2014). The renin-angiotensin-aldosterone system (RAAS) is central in controlling blood pressure under high salt conditions (Poch et al., 2001). Renin is responsible for cleaving angiotensinogen, which results in the production of angiotensin I (Ang I). Angiotensin converting enzymes (ACE) cleaves Ang 1 to make angiotensin II (Ang II) (Lavoie et al., 2003). Ang II is the physiologically active component of the system, and effects vasodilation, renal function, and the thirst center. The body expresses two types of receptors for Ang II known as AT-1 and AT-2. AT-1 receptors are responsible for mediating vasoconstrictor responses, where as AT-2 are responsible for vasodilator responses. AT-1 is mostly found in adults, where AT-2 is mostly found in fetuses. AT-1 is responsible for most of the physiological action of Ang II. Ang II binds to AT-1 to regulate renal sodium and water re-absorption and it stimulates the thirst center to increase fluid intake (Lavoie et al., 2003). Further, it stimulates the release of aldosterone from the adrenal glands.

1.3.3 Sodium and Endothelial Dysfunction

The effect of sodium on blood pressure is a well known pathway for sodium affecting the cardiovascular system (DGA, 2015; Wardener et al., 2004) More recent evidence, however, has shown that sodium has an effect on the endothelium, independent of blood pressure (DuPont et al., 2012; Greaney et al., 2001), which may be a precursor to development of atherosclerosis and high blood pressure and other forms of CVD. A study by DuPont et al., (2012) assessed endothelial function in salt-resistant adults after 7 days of both low sodium diet and high sodium diet. They showed a reduction in brachial artery FMD on the high sodium diet as compared to the low sodium diet indicating endothelial dysfunction. These findings support the idea that sodium in the diet can alter endothelial function independent of a change in blood pressure.

A decrease in sodium intake from 9g/day to 6g/day for 6 weeks showed improved endothelial function in overweight/obese individuals (Dickinson et al., 2014). Furthermore, this observation was characterized by a reduction in endothelin-1, a vasoconstrictor. Another study by Dickinson et al. (2009) demonstrated that two weeks of a high salt diet of 150 mmol/day decreased FMD compared to a low salt diet (50 mmol/day). Thus indicating that a high salt diet can cause endothelial dysfunction, independent of changes in blood pressure. These studies collectively suggest that high sodium diets cause endothelial dysfunction that can lead to hypertension and other forms of CVD while lower sodium diets improve endothelial function. Given that sodium consumption is controllable, by lowering dietary intake, risks for developing

CVD or endothelial dysfunction may be attenuated (Jablonski & Racine et al., 2013).

The mechanism responsible for sodium induced endothelial dysfunction has been suggested to be due to oxidative stress (Heitzer et al., 2001). It is proposed that high sodium intake leads to elevated levels of oxidative stress, specifically $O_2^{\bullet-}$, which decreases the bioavailability, and production, of NO by uncoupling BH4, thus decreasing NO in the endothelium resulting in endothelial dysfunction (Cai et al., 2000; Harrison et al., 2007; Ketonen et al., 2008; Oharah et al., 1993). Diets low in sodium and high in potassium and other minerals have been shown to lower oxidative stress and lessen cardiovascular damage (Hummel et al., 2012). High levels of $O_2^{\bullet-}$, and less NO lead to higher sodium reabsorption in the kidney and alter renal tubular feedback that can cause sodium/volume dependent hypertension (Wilcox et al., 2005).

While less work has been done to determine the mechanism of sodium's deleterious effect on the vasculature, infusion of ascorbic acid, a nonspecific antioxidant, has been shown to attenuate the effects of a high sodium diet on cutaneous vasodilation suggesting a role for oxidative stress through reduction of NO (Greaney et al., 2012; Heitzer et al., 2001).

1.4 Potassium

Potassium is a crucial mineral in the body, needed for electrolyte balance, body fluid volume, muscle contraction, nerve function, and normal metabolic functioning. Potassium can be found in many fresh fruits and vegetables and is low in processed foods. Decreased consumption of potassium

has been associated with increased risk of CVD, and increased consumption has been associated with having a protective effect against developing hypertension (Aburto et al., 2013). While high potassium diets have been shown to have BP lowering properties (Hoy et al., 2012), less is known about their effect on the vasculature independent of blood pressure.

1.4.1 Potassium recommendations

In the United States, the recommended intake of potassium is 4,700mg per day, which no countries, including the U.S. achieve (DGA, 2015). In 2009-2010, the average intake for people 2 years and older was 2,640 mg per day (Cogswell et al., 2012). Potassium intake tracks with energy intake, therefore, individuals consuming more energy per day generally consume more potassium (Hoy et al., 2012). Deficiency has been linked with increased blood pressure and increased salt sensitivity, as well as symptoms of hypokalemia that include muscle weakness and glucose intolerance (WHO, 2012). Low intake, especially when sodium intake is high, can lead to development of high blood pressure (WHO, 2012).

1.4.2 Potassium in the Body

Potassium intake has been positively correlated with improved endothelium-dependent vasodilation, as assessed by FMD, in those with hypertension (Blanch, Clifton, & Peterson et al., 2015). Blanch et al. (2014) demonstrated that consuming 2-3 servings of fruits and vegetables high in potassium can improve vascular function in one week as seen by an attenuation in postprandial brachial artery FMD. In a human study on individuals with salt-sensitive hypertension, after giving subjects the Dietary Approaches to Stop

Hypertension (DASH) diet for 21 days, a reduction in blood pressure, arterial stiffness, and oxidative stress, as measured through urinary F2-isoprostanes, was observed (Hummel et al., 2012). These studies help provide evidence to show that increasing potassium intake can attenuate some of the risk factors associated with CVD.

1.4.3 Ratio of Sodium and Potassium

High sodium to potassium ratios are associated with an increased risk for CVD (Cook et al., 2009). A dietary intervention study by Sacks et al. (2009), showed that use of the DASH diet, which is low in sodium and high in potassium lowered blood pressure in hypertensive individuals. Hypertension is associated with endothelial dysfunction, possibly the result of this dysfunction (Sacks et al., 2009). For every 0.5 increase in the sodium/potassium ratio, in adults 20+ years, examined in the 2005-2010 NHANES (Zhang et al., 2012) there was an increase of 1.05 mmHg in systolic blood pressure. Blanch, Clifton, & Keough et al. (2015) examined at the ability of potassium to attenuate sodium's effect on the endothelium following one of 3 meals: control meal with both sodium and potassium being low, a high potassium/ high sodium meal, low potassium/ high sodium meal. FMD was measured prior to the meal, and 30, 60, 90, and 120 minute post-prandial. The diets were randomized, in this controlled cross-over study. The high potassium meal significantly attenuated the decrease in FMD seen with a high sodium meal. This study showed that just 3 servings of fruits high in potassium (60mmol) could attenuate the negative effects of a high sodium meal (65 mmol).

Perez et al., (2014) state that the sodium/ potassium ratio might be more important than the individual amounts of sodium and potassium consumed by an individual, since a low ratio is usually derived from people who consume a diet low in sodium and high in potassium. Furthermore, they state that the ratio is especially important in relation to blood pressure, and that a low sodium/ high potassium ratio is ideal for optimizing cardiovascular health.

1.5 Aims and Hypotheses

Given the importance of diet as a non-invasive, inexpensive option to lower CVD risk, studying the impact of habitual dietary intake of sodium and potassium on endothelial function is important. Therefore, the following aims and hypotheses are proposed:

Aim one: Determine the relationship between habitual sodium and potassium intake on vascular function in healthy adults.

Hypothesis one: High potassium/low sodium diets will result in greater endothelial-dependent dilation as determined by brachial artery FMD and NO-mediated cutaneous vasodilation in response to local heating in healthy adults.

Aim two: Determine the relationship between habitual sodium and potassium intake on oxidative stress in the microvasculature in healthy adults.

Hypothesis two: High sodium/low potassium diets will result in an improvement in cutaneous vasodilation in response to infusion of antioxidants compared to subjects with a higher potassium/lower sodium diet.

Chapter 2

METHODS

2.1 Subjects

Subjects, both men and women, between the ages of 22 and 60 years were recruited through flyers and University of Delaware classifieds to participate. Subjects were excluded for a baseline blood pressure equal to or greater than 140/90, diagnosed or taking medication for hypertension or hypotension, heart disease, diabetes, kidney disease, obesity (BMI $\geq$ 30), tobacco use, pregnancy, or menopause. Those with strict dietary regulations were also excluded including those with celiac disease, vegans, vegetarians, gluten sensitivity, or eating disorders.

2.2 Screening

Twelve subjects were screened and three were excluded due to being post-menopausal. Subjects signed an informed consent (Appendix A), filled out a medical history questionnaire, and underwent a screening visit by a Nurse Practitioner at the Nurse Managed Health Center (NMHC) in the STAR Health Sciences Complex at the University of Delaware. A fasting blood sample was taken and analyzed for measures of liver function, a lipid profile, full blood count, glucose, sodium, potassium, chloride, and kidney function. Waist and hip circumference were also measured and body fat was assessed using a Tanita Scale.

2.3 Collection of Habitual Dietary Information

Subjects were asked to complete a 3-day diet to record habitual data prior to their visits. Subjects also completed a 24-hour dietary recall the day they underwent vascular testing to compare their sodium intake on that day to their urinary sodium excretion. The data were entered into the Nutrition Data System for Research program from the University of Minnesota, Minneapolis, MN, 2015 (NDSR). Each day was analyzed for energy, macronutrients, micronutrients, alcohol, and caffeine.

2.4 Vascular Testing Day

Prior to the study visit, subjects were asked not to eat for 4 hours prior, not to drink alcohol or caffeine for 12 hours prior, and not to exercise for 24 hours prior to the visit. The visit lasted 4-5 hours. Upon arrival to the STAR cardiovascular lab, subject's height and weight were taken. For measurement of height, subjects stood straight, with no shoes or socks, against a stadiometer (Health O Meter, McCook, IL). Subjects stood barefoot on a Tanita scale while it recorded their weight, body mass index (BMI), basal metabolic rate (BMR), and percentage of body fat.

2.5 24-Hour Blood Pressure and Urine Collection

On the morning of the visit, the subject began wearing a 24-hour ambulatory blood pressure monitor (Space Labs, Snoqualmie, WA). The blood pressure monitor inflated every 20 minutes while awake, and every 30 minutes while asleep. Subjects reported their wake and sleep times prior to the day they wore it, so times could be pre-programmed into the device. Subjects were instructed to remain as still as possible

and relax when they felt the cuff begin to inflate. The cuff was off during the study visit for the first hour, and then placed on them during the study visit when the assessment of cutaneous microvascular function testing began.

For the 24-hour urine collection, after waking time, subjects recorded the time and urinated in the toilet. Following this first void, subsequent voids were collected in the container, until the following morning when they woke up, recorded the time, and urinated one last time into the container. Subjects delivered both the blood pressure cuff and the urine back to the STAR complex at the University of Delaware the day after their visit to be analyzed.

2.6 Assessment of Cutaneous Microvascular Function

Subjects were instructed to lie down in a semi-recumbent position to assess microvasculature blood flow in response to localized heating. Using sterile techniques, four microdialysis fibers (CM 31 Linear MD Probe Fibers) with permeable membranes were inserted in the ventral side of the left forearm. Prior to insertion, the forearm was cleaned with three swabs of betadine and alcohol. It was then iced for 10 minutes. Four 23-gauge needles were inserted into the dermal layer on the forearm. Fibers were thread through the lumens of the needles, and the needles were removed, leaving the membrane of the fiber in place under the skin. The fibers were attached to four separate syringes located in a pump next to the bed, set at a speed of 2 microliters/min. Each site had a heater placed on top of it and taped down, with a laser

Doppler placed through the heater. All syringes were perfused with Lactated Ringers solution for a minimum of 60 min to allow the hyperemia to subside.

After the hyperemia subsided, the four microdialysis sites were randomly assigned to the following: 1) Lactated Ringers solution (control site); 2) ascorbic acid; 3) Tempol (a superoxide dismutase mimetic); and 4) apocynin (NAD(P)H oxidase inhibitor) (one control site and three antioxidants). After 10 minutes of a steady baseline recording of red blood cell flux (RBC flux) at 33°C, the heat was increased in increments of 0.5°C to 42°C to begin local heating. Local heating ran for a minimum of 20-30 minutes. Once the RBC flux has reached a plateau for 10 minutes, L-N^G-Nitroarginine methyl ester (L-NAME) a NO blocker, was perfused through all 4 sites and the pump speed was increased to 4 microliters/minute. After about 20-30 minutes of L-NAME infusion, once RBC flux has reached its lowest point, sodium nitroprusside (SNP) was perfused through the four sites and the heat was increased in increments of 0.2°C to 43°C. SNP ran for about 15-20 minutes until RBC flux reached its highest point.

2.7 Blood and Urine Analyses

During the study visit, a venous blood sample was drawn for the analysis of electrolytes, including potassium, sodium, and chloride in the blood and hematocrit (Clay Adams Brand, Readacrit Centrifuge; Becton Dickinson, Sparks, Maryland, USA), and hemoglobin (Hb 201p model; HemoCue, Lake Forest, Califonria, USA) in blood. Sodium, potassium, and chloride for urine were measured using the Medica

Easy Electrolyte Analyzer (Medica Corporation, Bedford, MA). Osmolality of blood and urine was measured using the Advanced Osmometer (Two Way Technology, Norwood, MA).

2.8 Brachial Artery Flow-Mediated Dilation (FMD)

Brachial artery FMD was performed on the right arm to assess responses to hyperemia. A 10 MHz linear phased array ultrasound transducer (Logic e, GE) was used to measure the blood flow and dilation of the brachial artery prior to, during and following cuff occlusion. Following one minute of baseline image recording, the cuff was inflated to 200 mmHg for five minutes. After five minutes, the cuff deflated and the brachial artery images recording continued for two minutes post deflation. Brachial artery FMD was used as a measure of endothelial dependent function and expressed as a percent change from baseline normalized for shear rate and calculated from the blood flow velocity and vessel diameter.

2.9 Statistics

The main variables of interest were percent change in brachial artery FMD and the NO-mediated plateau. The analysis sought to determine if a relationship exists between habitual dietary intake of sodium and potassium and FMD and the NO-mediated plateau. Pearson correlations were run between sodium intake, potassium intake, and sodium/potassium intake ratio and the main variables of interest. Secondly, a one-way ANOVA was performed to assess differences in the NO contribution to the plateau between the four sites. One way and two way ANOVAs were ran to see if

there were differences between baseline measurements and plateau measurements during assessment of cutaneous microvascular function. Graphpad Prism was used to run the statistical analysis. Significance was set at $P < 0.05$. All data are presented as mean ± standard error of the mean (SEM).

Chapter 3

RESULTS

3.1 Subject Characteristics

Nine subjects (5F/4M) completed the study. Seven subjects were Caucasian and two subjects were African American. Average age was 28.8 years with a range from 22-54. The average BMI was 25.4 kg/m^2, placing them just above normal range, into the overweight category. Body composition was assessed on their vascular testing day, and the average body fat was 27 ± 2.89% and fat mass was 45.76 ± 5.33 kg. Subjects had normal blood lipid profiles and renal function. More detailed demographics can be seen in Table 3.1.1.

Table 3.1 Subject Characteristics From Screening Data

Variables	Subjects (N=9)
Age (years)	28.8 ± 4
Mass (kg)	74.7 ± 3.2
Height (cm)	172.8 ± 2.7
BMI (kg/m^2)	25.4 ± 0.8
Systolic BP (mmHg)	120 ± 3
Diastolic BP (mmHg)	74 ± 2
Total Cholesterol (mg/dL)	170 ± 19
LDL Cholesterol (mg/dL)	94 ± 15
HDL Cholesterol (mg/dL)	59 ± 6
Triglycerides (mg/dL)	85 ± 18
Glucose (mg/dL)	81 ± 3
Blood urea nitrogen (mg/dL)	11 ± 1
Creatinine (mg/dL)	1 ± 0.07
eGFR (mL/min/1.73m2)	107 ± 5
Serum Sodium (mmol/L)	138 ± 0.33
Serum Potassium (mmol/L)	4 ± 0.09
Serum Chloride (mmol/L)	103 ± 1
Plasma Osmolality (mOsm/kg)	285 ± 1
Hemoglobin (g/dL)	13.7 ± 0.32
Hematocrit (%)	41.19 ± 1

BMI: Body Mass Index; BP: Blood Pressure; eGFR: estimated glomerular filtration rate; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein. All data is shown as mean ± SEM.

3.2 Dietary Data

All subjects completed a 3-day diet record prior to starting the study. Average energy intake was 2113 kcal/day with a range of 1446 to 3121kcals. The percentage of energy from carbohydrates was 44%, from protein was 16% and from fat were 38%. Our men consumed less than the average American for all macronutrients, and our women consumed less for carbohydrates and protein, but consumed more for fat.
Subjects on average consumed 3586 mg/day of sodium with a range of 2322 to 5171 mg and 2516 mg/day of potassium with a range of 1865 to 3308 mg. The ratio of habitual sodium and potassium ranged from 1.05 to 1.81, while the average ratio was

1.42 - falling well above the recommended ratio of 0.49. Dietary intake for key nutrients can be seen in Table 3.2.1. For more detail on their dietary intake, please see Appendix C.

Table 3.2 Three Day Diet Record Data – Habitual Intake

Variables	Subjects (N=9)
Energy (kcal)	2113 ± 200
Total Carbohydrate (g)	233 ± 30
Total Protein (g)	85 ± 8
Total Fat (g)	88 ± 10
Cholesterol (mg)	324 ± 54
Sodium (mg)	3586 ± 396
Potassium (mg)	2516 ± 138
Sodium to Potassium Ratio	1.42 ± 0.13
Alcohol (g)	9 ± 4
Caffeine (mg)	83 ± 25
Water (g)	1665 ± 271

Values are mean ± SEM.

A 24-hour diet record was taken on the day of laboratory testing to compare subject's intake of sodium and potassium to urinary excretion values. Average dietary sodium and potassium intake on the testing day can be seen in Table 3.2.2. Both sodium and potassium intakes were higher on the 24-hr recall compared to the 3-day diet record however; data were only collected on 5 subjects. One subject never returned the diet record and three subjects were enrolled prior to the start of collecting these data. While consuming caffeine and alcohol was not allowed during the vascular-testing day, one subject did consume 46 mg, and one subject consumed 0.29

g alcohol, accounting for the caffeine and alcohol intake. More detailed dietary data from the 24-hour recall can be seen in Appendix C.

Table 3.3 24-Hour Recall – Vascular Testing Visit

Variables	Subjects (N=5)
Energy (kcal)	2247 ± 233
Total Carbohydrate (g)	258 ± 22
Total Protein (g)	103 ± 21
Total Fat (g)	94 ± 18
Cholesterol (mg)	466 ± 132
Sodium (mg)	4279 ± 82
Potassium (mg)	2645 ± 489
Sodium to Potassium Ratio	1.77 ± 0.32
Alcohol (g)	0.29 ± 0.28
Caffeine (mg)	46 ± 46
Water (g)	2213 ± 304

Values are mean ± SEM.

3.3 Blood Pressure Data

Ambulatory blood pressure was collected on all subjects for 24 hours starting on their vascular testing visit day. All subjects were normotensive and fell within expected ranges (see Table 3.3.1).

Table 3.4 24-hour Ambulatory Blood Pressure Data

Variables	Subjects (N=9)
Systolic Blood Pressure (mmHg)	116 ± 3
Diastolic Blood Pressure (mmHg)	69 ± 2
Mean Arterial Pressure (mmHg)	85 ± 2
Pulse Pressure (mmHg)	49 ± 4
Heart Rate (BPM)	64 ± 4

Values are mean ± SEM.

3.4 Biochemical Data

Blood analysis was completed during the study visit for all 9 subjects and is shown in Table 3.4.1. All values fell within the normal range. While there is some variability between the screening data and vascular testing day data, no statistically significant differences were found. It should be noted that the samples from the screening visit were sent to an outside lab for analysis, while the vascular testing day visit bloods were analyzed in our lab. Table 3.4.1 outlines the blood values in more detail.

Table 3.5 Blood Data

Visit Day Blood Data	Subjects (N=9)
Plasma Osmolality (mOsm/kg)	282 ± 2.4
Serum Sodium (mmol/L)	137 ± 1.3
Serum Potassium (mmol/L)	4.02 ± 0.06
Serum Chloride (mmol/L)	103.5 ± 1.2
Hematocrit (%)	39.5 ± 1.1
Hemoglobin (g/dL)	13.1 ± 0.4

Values are mean ± SEM.

Urine analysis was also completed during the study visit for all 9 subjects. Average urinary sodium excretion over 24 hours was 155 mmol/L and potassium excretion was 50.5 mmol/L and is shown in Table 3.4.2. Dietary intake data from the 24-hour recall shows the subjects consumed 4279 mg (186 mmol/L) sodium and 2645 mg (68 mmol/L) potassium. Comparison between the 24-hour recall and excretion can be seen in Figure 3.1. The r-values for the graphs in Figure 3.1 are not significant although they are only based on 5 subjects.

Table 3.6 Urine Data

Variables	Subjects (N=9)
Urine Osmolality (mOsm/kg H_2O)	464.33 ± 250.02
24hr Sodium Excretion (mmol/L)	160.5 ± 51.88
24hr Potassium Excretion (mmol/L)	55.46 ± 21.05
Urine Volume (mL)	2033.33 ± 923.63
Urine Specific Gravity	1.01 ± 0.00

Values are mean ± SEM.

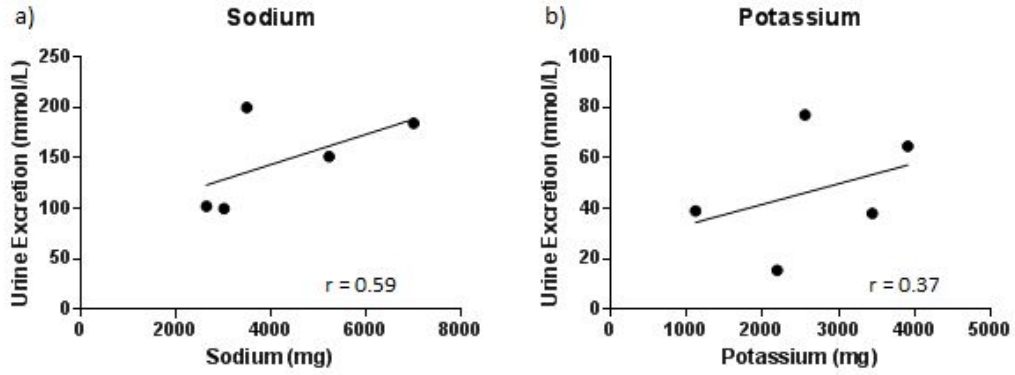

Figure 3.1. a) Comparison of urinary sodium excretion and 24 hour recall data, and b) comparison of urinary potassium excretion and 24 hour recall data.

3.5 Microvascular Function

Microvascular function data is shown in Table 3.5.1 and Figures 3.2 and 3.3. Of nine subjects, all underwent assessment of cutaneous microvascular function, however only seven subjects received all three antioxidants, due to a heater issue; two subjects did not receive ascorbic acid. Table 3.5.1 provides the average data for each

of the four sites for baseline, initial peak, the NO plateau, L-Name plateau and the NO contribution. Figure 3.2a shows that there was no difference among the sites for baseline %CVCmax ($P > 0.05$). Figure 3.2b shows that the NO-mediated plateau did not differ between sites either ($P > 0.05$).

Correlations were run between the NO plateau and dietary intake of sodium and potassium as well as the ratio for each of the antioxidants infused at the three sites. There was no correlation between the plateau phase for any antioxidant with potassium or with the sodium to potassium ratio. However, there was a significant correlation between sodium intake and the ascorbic acid site ($R = 0.7650$; $P < 0.05$; Figure 3.3). Correlations were also run using the urinary excretion values for sodium or potassium and the NO plateau. Similar to the results from the intake correlations, no significant relationship was found with the NO mediated plateau.

Table 3.7 Assessment of cutaneous Microvascular Function data.

Time point	Ringers (n=9)	Ascorbic Acid (n=7)	Apocynin (n=9)	Tempol (n=9)
Baseline	8.66 ± 0.91	10.83 ± 2.25	10.64 ± 2.74	10.81 ± 3.38
Initial Peak (IP)	90.67 ± 2.04	94.17 ± 1.08	92.81 ± 2.37	91.28 ± 2.80
Nadir (ND)	71.07 ± 6.27	78.94 ± 3.28	81.82 ± 5.65	77.92 ± 6.66
Plateau	57.75 ± 9.21	73.25 ± 3.77	62.09 ± 11.47	73.76 ± 5.91
L-Name Plateau	19.83 ± 6.12	42.25 ± 7.22	38.44 ± 11.67	30.16 ± 8.71
NO Contribution	70.84 ± 5.80	51.91 ± 7.23	54.37 ± 10.76	61.12 ± 7.20

Data are mean +/- standard error of the mean; CVC: Cutaneous vascular conductance; L-NAME: L-Nitroarginine methylester; NO: Nitric Oxide

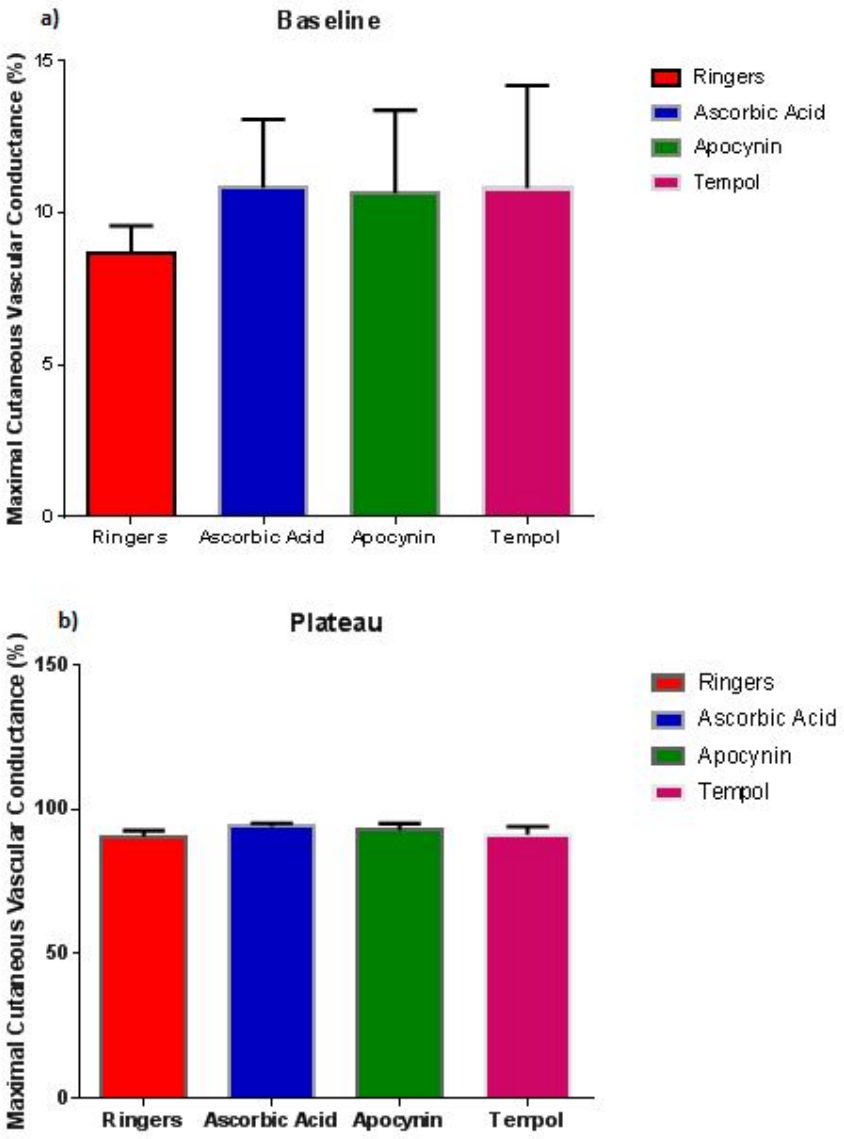

Figure 3.2. The percentage of maximal Cutaneous Vascular Conductance for all sites for (a) baseline and (b) the plateau phase.

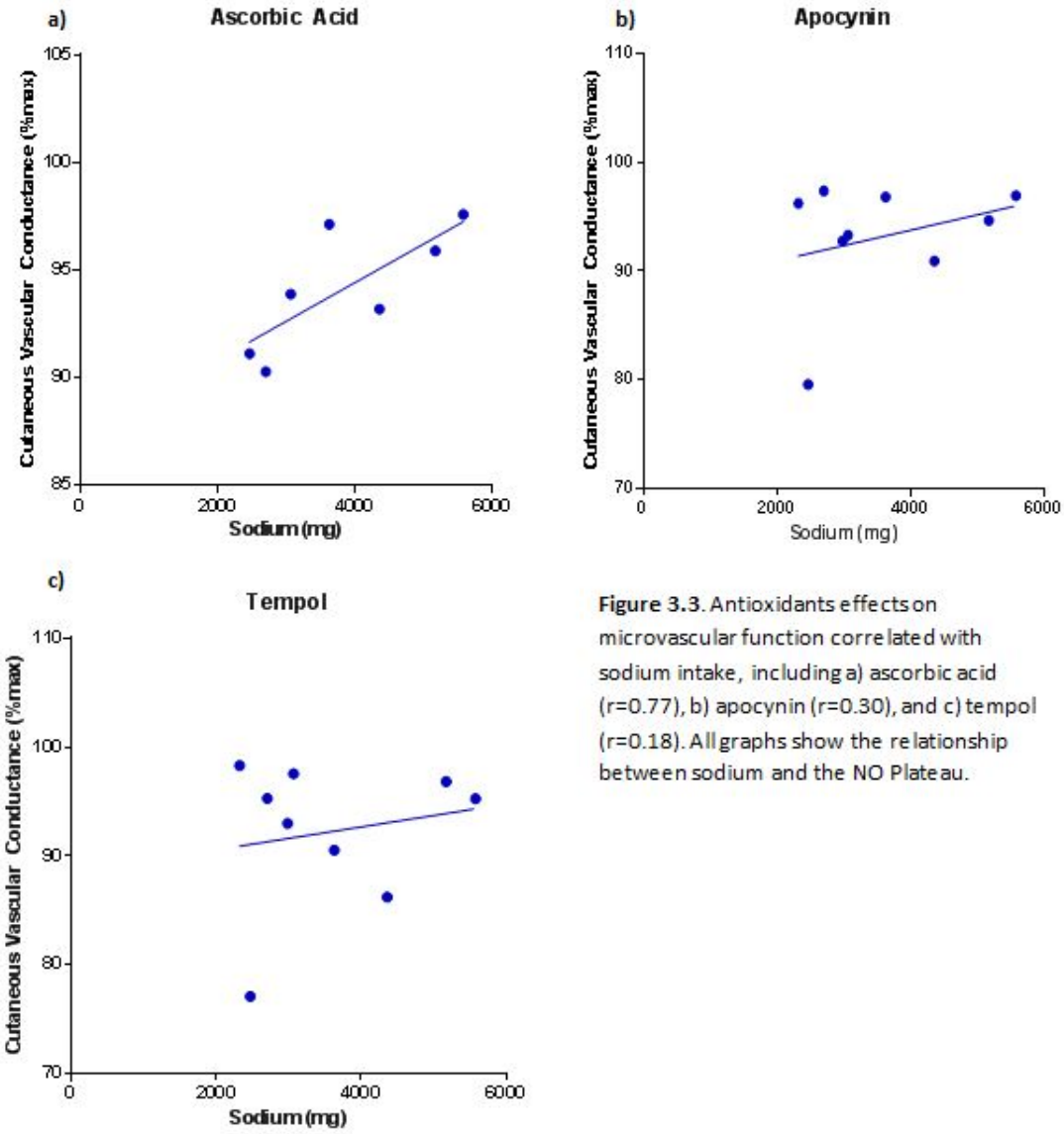

Figure 3.3. Antioxidants effects on microvascular function correlated with sodium intake, including a) ascorbic acid (r=0.77), b) apocynin (r=0.30), and c) tempol (r=0.18). All graphs show the relationship between sodium and the NO Plateau.

3.6 Flow Mediated Dilation (FMD)

Brachial artery FMD was completed on eight subjects (5F/3M), as one subject's data was not analyzed due to movement during the data collection, and hence, distortion in the video. The FMD data is shown in Table 3.6.1. The percent

change in diameter ranged from 2.5-12.8%, with an average of 6.24%, which is considered a normal value for a healthy population.

Table 3.8 Brachial Artery Flow-Mediated Dilation data.

Variables	Subjects (N=8)
Baseline Brachial Artery Diameter (cm)	0.35 ± 0.02
Peak Brachial Artery Diameter (cm)	0.38 ± 0.02
Brachial Artery FMD, Δ cm	0.021 ± 0.003
Percent Change Diameter (%)	6.24 ± 1.26
TTP Diameter (s)	39.49 ± 6.13
Shear, AUC	20,036 ± 5,003

Values are mean ± SEM. AUC: Area under the curve; TTP: Time to peak

Correlations were run to determine if there was relationship between dietary intake of sodium, potassium, and the sodium to potassium ratio with FMD. The correlations are shown in Figure 3.4. There was no significant correlation between any of these dietary nutrients and FMD, as well as any of the excretion values for these nutrients and FMD. In particular, the best-fit line for the potassium graph shows that lower potassium intakes are associated with higher FMD, which was unexpected ($R = -0.53$; $P > 0.05$). For sodium, as dietary intake increased, FMD declined as expected although this was not significant ($R = -0.49$; $P > 0.05$). A similar relationship was seen for the sodium to potassium ratio and FMD ($R = -0.21$; $P > 0.05$). There was no significant relationship between 24 hour urinary excretion values for sodium or potassium with any of the FMD measurements.

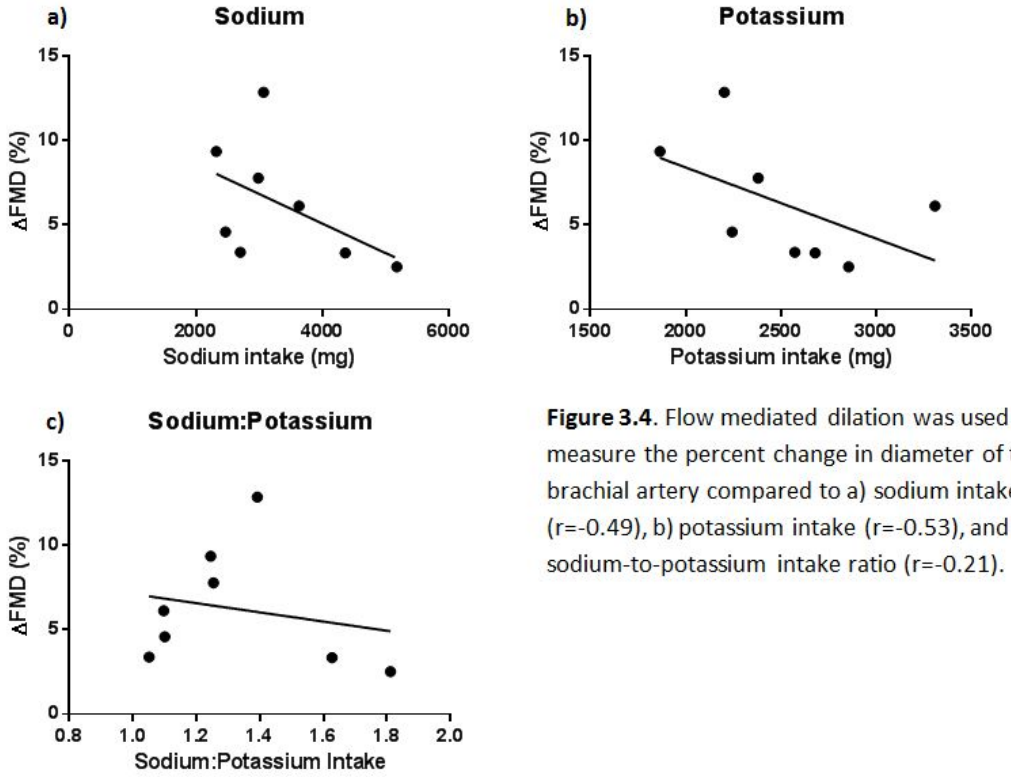

Figure 3.4. Flow mediated dilation was used to measure the percent change in diameter of the brachial artery compared to a) sodium intake (r=-0.49), b) potassium intake (r=-0.53), and c) sodium-to-potassium intake ratio (r=-0.21).

Chapter 4

DISCUSSION

The purpose of this study was to determine the effect of habitual sodium and potassium intake on vascular function in healthy, normotensive adults. We hypothesized that a diet low in sodium and high in potassium would result in greater endothelial-dependent dilation as determined by brachial artery FMD and NO-mediated cutaneous vasodilation in response to local heating in healthy adults compared to a diet high in sodium and low in potassium. We also hypothesized that a diet low in potassium and high in sodium would result in an improvement in cutaneous vasodilation in response to infusion of antioxidants compared to subjects with a higher potassium/lower sodium diet. The primary findings of this study were 1) there was no correlation between brachial artery FMD and dietary intake of sodium, potassium, or the sodium to potassium intake ratio, and 2) infusion of ascorbic acid was related to improved NO-mediated plateau in subjects with a higher sodium intake. In relation to the FMD data, the best-fit line did show a downward trend with sodium, and with the ratio, as expected, and perhaps with more power, the correlation coefficient would increase. However, potassium also showed a downward trend, which was unexpected. With regards to microvascular function, the study revealed no relationship between sodium, potassium, or the ratio of sodium-to-potassium with improvements in cutaneous vasodilation in response to infusion of antioxidants, except for ascorbic acid and sodium, suggesting that oxidative stress is present in those with a higher sodium intake.

4.1 Dietary Intake

During our study, subjects were asked to record their dietary intake for 3 days prior to the vascular testing visit. They also filled out a 24-hour recall on the day of the vascular testing visit to compare their dietary intake of sodium and potassium with their urinary excretion of these two minerals. The 3-day diet records revealed that consumption of all the macronutrients fell within the acceptable macronutrient distribution ranges (AMDR). However, fat tended to be on the high end of the AMDR with four subjects exceeding it with over 40% of their energy from fat. Overall, our men consumed less than the average American for all macronutrients, and our women consumed less for carbohydrates and protein, but consumed more for fat.

In our study, men consumed an average of 2,205 kcalories while the women consumed 1,980. This is a little below the recommended intake of 2,800 for men and of 2,000 for women of a similar age range who are moderately active (USDA, 2016). Data analysis revealed similarities between the dietary intake of our subjects to NHANES data from 2005 to 2010 on 10,563 subjects who were not on blood pressure medication or following a low sodium diet (Zhang et al., 2012). The NHANES data showed that the average U.S. adult exceeds the AI for sodium and does not meet the AI for potassium. Indeed, on average, American's intake of sodium greatly exceeds the AI by 229% while potassium intake falls short at 50% of the AI (DGA, 2015). Our subject's dietary records mirror this pattern. Our subject's sodium intake was 239% above the AI and 53% of the AI for potassium. When looking at the sodium to potassium intake ratio, it was 1.41 in our subjects and this matches what the NHANES national data shows (Zhang et al., 2012).

4.2 Biochemical Parameters

The blood and urinary variables collected during this study were all found to be in the normal range. Those blood variables that were measured more than once were consistent over time. The urinary sodium and potassium excretion values were slightly lower than expected, based on 24-hour recall data. The discrepancy in recall data compared to urinary excretion data could be caused by over reporting in the diet recall or if a subject missed a urine collection and did not report it. Since there was only data on 5 subjects for the 24-hr recall, it is likely that this relationship would be significant with a few more subjects. In regards to the blood pressure data, all subjects were normotensive during their laboratory blood pressure measurements and during the 24-hour monitoring.

4.3 Antioxidant Effect on Cutaneous Microvascular Function

Cutaneous microvascular function has been used as an accessible vascular bed to study endothelial function. Further, it has been shown to be a good predictor of cardiovascular outcomes (Holowatz et al., 2008) and can be used to assess endothelial function (Ijzerman et al., 2003). Further, studying the microvasculature has been shown to be a minimally invasive and effective technique to assess the vascular system, since the micro vessels appear to mirror the larger vessels (Ijzerman et al., 2003). Finally, the cutaneous circulation is commonly used to study the microvasculature to gather pre-clinical data on CVD and monitor disease progression (Holowatz et al., 2008).

While there are limited studies evaluating the effect of sodium intake on microvascular function, a study by Greaney et al., (2012), examined cutaneous microvascular function following 7 days of low sodium (20mmol/day) and 7 days of a high sodium diet (350 mmol/day) in12 normotensive adults with an average age of 31 years. They found that the NO plateau was significantly reduced during the high sodium diet (80 ± 2%) while a low sodium diet improved the plateau (93 ± 1%). Perfusion with ascorbic acid, a non-specific antioxidant, improved the plateau on the high sodium diet compared to ringers (Ringers: 80 ± 2% vs. AA: 89 ± 3%, $p<0.05$). Their overall conclusion was that high salt diets can impair microvascular function even without changes in blood pressure and ascorbic acid can attenuate these negative effects suggesting a role for oxidative stress.

Oxidative stress is thought to be a potential mechanism for the deleterious effects of high sodium on the vasculature. The Greaney et al., (2012) study demonstrated that ascorbic acid improved endothelial function under high salt conditions. Similar to this study, we have shown that those subjects with higher sodium intakes had an improved NO-mediated plateau in the presence of ascorbic acid compared to subjects with less sodium in the diet. This improvement was not seen with administration of tempol or apocynin, two other antioxidants. It is likely that we are underpowered to see an effect. Other studies assessing cutaneous microvascular function have also shown that oxidative stress is present and infusion of antioxidants can improve vasodilation (Alexander et al., 2013; DuPont et al., 2014). In a study by

Alexander et al., (2013), infusion of BH4 in hypercholesterolemic subjects increased NO vasodilation through the NOS coupling mechanism. BH4 has been shown to have antioxidant capabilities. Finally, DuPont et al., (2014) demonstrated that patients with CKD had worse endothelial function than age matched controls that was improved with infusion of tempol and apocynin. Subjects in this study were individuals with stage 3 - 4 kidney disease. This again provided evidence that oxidative stress plays a role in endothelial dysfunction. While this present study only showed a relationship between high sodium diets and an improvement in the NO plateau by local infusion of ascorbic acid, likely the addition of more subjects would show a similar relationship in the tempol and apocynin sites.

4.4 Endothelial Function

We assessed endothelial function in subjects on their habitual diet to determine whether individuals with higher intakes of sodium and lower intakes of potassium corresponded with poorer endothelial function. The average percent change in FMD for our subjects was 6.23% that is consistent with other published studies (Jablonski et al., 2013).

Marginal correlations were found between the dietary intake of sodium, potassium. This was expected for sodium and the sodium to potassium intake ratio. As sodium increased, we expected to see a correspondingly lower FMD value. This association has been shown previously shown in a controlled feeding study by Dupont et al., (2014). They demonstrated that subjects on a high sodium (HS) diet had a

reduction in FMD compared to a low sodium (LS) diet (HS: 7.3±0.7% vs. LS: 10.3±0.9%). Our correlation between our subject's sodium intake and FMD revealed an R of -0.49 that did not reach statistical significance. The weakest correlation was seen between the intake ratio and FMD (R = -.021). However, the strongest correlation was for dietary potassium with an R value of -0.53. This trend was unexpected, as it showed that the more potassium consumed, the lower the percent change in FMD. This may have happened due to in accurate reporting of dietary intake, low subject number, or for not controlling for menstrual cycle, which can alter FMD results (Williams et al., 2001).

The influence of sodium and potassium on FMD has been studied primarily in diet-controlled studies, not while subjects are on their habitual diet. Jablonski et al., (2014) looked at 17 subjects with an average age of 62 who completed 4 weeks of a dietary salt restriction (DSR) diet and a normal sodium diet. They saw that the DSR diet group had a 68% higher percent change in FMD (6.01 ± 2.31% vs. 3.57 ± 1.69%) than the normal salt. While we did not control our subject's sodium intake, we do see that, in general, those subjects with a higher sodium intake had a lower FMD and that those with a lower sodium intake had a higher FMD which follow the results of this study. However, there are some differences as the average FMD % in our subjects was 6.23%, which is similar to the FMD seen in the DSR group that consumed a much lower amount of sodium (1,200mg/day) than our subjects. Indeed, our subject's average sodium intake (3586 mg/day) more closely represents the normal group

(3,600mg/day). It should be noted that the study population in the Jablonski study was much older and had moderately elevated blood pressure, which may account for their lower FMD on the normal sodium diet. This observation was also seen similarly in another study on obese and overweight normotensive men and women (Dickinson et al., 2009). They found that FMD was improved on a low salt (50mmol/day = 1150mg) compared to a usual salt diet (150mmol/day = 3,450mg). Reported FMDs were 4.89 ± 2.42% and 3.37 ± 2.10%, respectively. The usual salt diet in this study is similar to the habitual sodium intake recorded in this study. However, their average FMD for the usual sodium group is lower than the findings in this study by ~2%. This association could be due to the fact that the subjects were overweight/obese individuals, where as this study focused on those with normal BMI. Further, their average subject was much older than this study at 51.2 ± 6 years while our study was 28.8 ±84 years.

Although sodium is known to have a negative effect on FMD, potassium has been shown to have a positive effect (Blanch et al., 2015). In an acute study by Blanch et al., (2014), subjects were given either a high potassium (36mmol) or a low potassium (6mmol) food and FMD was measured at 30, 60, 90, and 120 minutes post prandial and compared to baseline. They showed that a high potassium food similar to what is found in 2.5 bananas was able to significantly increase FMD. According to the USDA food composition database, 2.5 bananas contain 1217mg of potassium. The AI for potassium is 4,700 mg/day; therefore, this study showed that acutely consuming just 1/3 of the daily potassium AI can increase FMD in the postprandial state. In the

current study, the average potassium intake was 2423 ± 112 mg/day, which means according to findings by Blanch et al., 2015 that this should be enough to help prevent a postprandial decline in their FMD. A second study by Blanch et al., (2014b) looked at the chronic effects of potassium on FMD after a week of high potassium diet and a week of a low potassium diet in 35 healthy men and women. They saw a significant increase in FMD (0.6% ± 1.5%) in the high potassium group when compared to the low potassium diet group. They conclude that a high potassium diet is beneficial for the vasculature but state the mechanism remains unclear. In our study, we saw no significant relationship between sodium, potassium, or the ratio and FMD. In fact, in this study there was a decrease in FMD as dietary potassium intake increased. At this time, it is unclear why this occurred but likely with an increase in subject number, this would change. Finally, it may be due to over-reporting as stated earlier. Clearly, the evidence presented from other studies does not support this finding, as a diet rich in potassium appears to improve FMD even in the presence of sodium.

A high sodium to potassium ratio is associated with an increased risk for CVD (Cook et al., 2009). The ratio is important to look at as it has been shown to be related to changes in blood pressure. Further, there appears to be a stronger relationship between the ratio and CVD than looking at sodium or potassium alone. Zhang et al., (2012) reported that for every increase of 0.5 in the ratio, there was an increase of 1.05mmHg in systolic blood pressure. Gijsbers et al., (2015) looked at FMD in response to differing amounts of sodium and potassium intake in older pre-

hypertensive and hypertensive adults (average age 65.8 ± 8.8 years). All subjects were given a diet consisting of 2.4 g/day of sodium and 2.3 g/day of potassium. They were randomized into 3 groups and given a supplement to take throughout the study in addition to the fixed amounts of dietary sodium and potassium listed above. The supplements included 3.0 g sodium, 2.8 g potassium, or a placebo. This resulted in a sodium to potassium ratio of 2.34 (on the sodium supplement), 0.48 (on the potassium supplement), or 1.04 (on the placebo). When FMD was measured at the end of the study, the placebo group had a FMD of 2.85 ± 2.83% and with the addition of the potassium supplement, an increase in FMD to 4.04 ± 1.82%. Our subjects had a ratio of 1.42 that is most similar to the placebo however our FMD values are much higher. This study did have significantly older subjects than our study, and included hypertensive subjects as well. Further, we saw no relationship between the ratio and FMD. This may be driven by the potassium intake of our subjects and also by the limited number of subjects in our study.

4.5 Limitations

There are several limitations to the current study. One, this is a pilot study with only 9 subjects so we are underpowered to truly answer our questions. Two, collection of accurate dietary data is challenging and subjects often under- or over-report consumption. We did correlate dietary intake with the urinary excretion of sodium and potassium and saw a positive correlation that would be stronger with more subjects. Also, two subjects consumed substances including caffeine, and alcohol during the 24-

hour urine and blood pressure collection, which could have led to skewed result. Further, using 3 days as a reflection of habitual dietary intake is a very short period of time and may not reflect their actual intake. Food frequency questionnaires or a longer recall, such as multiple 3-day diet records may be more appropriate and could potentially give a better picture of the diet. Third, were unable to get cutaneous microvascular function data at our ascorbic acid site in two subjects due to an equipment issue. Fourth, we were unable to analyze FMD on one subject due to excessive movement during the ultrasound. Hence, we have several missing data points due to experimental failure. Fifth, the habitual data revealed all subjects consumed more sodium than potassium, and no subject reached the AI for potassium, which limited one of they aims of this study; that is, having subjects who consume a low sodium/high potassium intake.

4.6 Future Directions

A future study should include more subjects to truly answer this question. Future studies should include multiple 3-day diet records to give a more clear idea of habitual diet, instead of just one 3-day record. Subjects may also benefit from one-on-one training before dietary data collection to improve accuracy of reporting. Further, more frequent reminders on not consuming banned substances (i.e. alcohol, caffeine) during visits may help.

4.7 Conclusion

In conclusion, the habitual intake of sodium and potassium had no effect on the vascular system of healthy adults. It is evident with previous research and the results of this study that more power would probably have yielded significant results.

The main finding of this study was a correlation between sodium intake and the NO-mediated plateau at the ascorbic acid site during assessment of microvascular function. This finding showed that ascorbic acid successfully attenuated the negative effects of sodium on the vascular system in the skin microvasculature and supports previous research suggesting that a high sodium diet increases oxidative stress. This supports the hypothesis that oxidative stress is responsible for endothelial dysfunction under high sodium conditions. However, based on our data, potassium is not playing a protective role in the vasculature.

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Appendix A

INFORMED CONSENT

UD IRB Approval from 05/11/2015 to 07/16/2015

INFORMED CONSENT

Title of Project:	Vascular Effects of Dietary Potassium in Humans
Principal Investigator:	Shannon Lennon-Edwards, PhD, RD
Other Investigators:	William Farquhar, PhD; Dave Edwards, PhD; Karen Solecki, BS; Beth Nachman, BS; Katherine Masso, BS; Stephanie Mraz, BS

SUBJECT NAME: _____

You are being asked to participate in a research study. This form tells you about the study including its purpose, what you will do if you decide to participate, and any risks and benefits of being in the study. Please read the information below and ask the research team questions about anything we have not made clear before you decide whether to participate. Your participation is voluntary and you can refuse to participate or withdraw at anytime without penalty or loss of benefits to which you are otherwise entitled. If you decide to participate, you will be asked to sign this form and a copy will be given to you to keep for your reference.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to look at the effects of a high and low potassium diet in the presence of controlled sodium (i.e. salt) intake on blood vessel function. The aim of this study is to determine if a high potassium diet is protective against the effects of a high sodium diet on the ability of your blood vessels to dilate (widen).

You will be 1 of 45 subjects (men and women) between the ages of 22 and 60 years old who will be recruited for this pilot study. All subjects recruited will have either a normal blood pressure or "high normal" blood pressure (this has more recently been termed "prehypertension"). You may be excluded from volunteering if you have high blood pressure (i.e. hypertension), medically diagnosed hypotension with symptoms, known heart disease, diabetes, kidney disease, are a smoker, are pregnant, obese (BMI>30) or are on any medications for those conditions. You may also be excluded if you have very specific dietary restrictions such as celiac disease, gluten sensitivity, are vegetarian, vegan, or indicate you have an eating disorder.

Full participation in this study involves completion of 2 phases: (#1) subject screening, (#2) testing of blood vessel function. These 2 phases will occur over a 6-week period, and will include a total of not more than 9 separate visits to the Vascular Physiology Lab (total time is approximately 21 hours) at the University of Delaware STAR Campus, 540 S. College Ave., Newark, DE. Each phase is outlined below.

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WHAT WILL YOU BE ASKED TO DO?

1. Subject Screening (1 visit about 1 hour in length)

We ask that you avoid caffeine, alcohol, and exercise in the 12 hours prior to this visit. In order to determine if you qualify to be in the study, the following information will be obtained: a complete medical history using a questionnaire, height, weight, waist circumference, hip circumference, a resting electrocardiogram (ECG: 10 self-adhesive electrodes will be placed on your chest), resting blood pressure, a body fat assessment, and a blood sample (obtained with a needle from a vein in the arm). The physical exam, ECG, blood and urine sample will be collected by a licensed Nurse Practitioner in the NMHC. The blood sample will be used to obtain an assessment of liver function, a lipid profile, a complete blood count, glucose, sodium, potassium, chloride, and kidney function. About 2 teaspoons will be sampled. All information will be reviewed by the investigators, and only those with no signs or symptoms of disease, blood work within normal limits, and a resting electrocardiogram within normal limits, will be accepted into the study. The NMHC will let the investigators know if you are medically cleared to participate. It will take several days to get the results of your bloodwork and therefore you cannot be cleared to participate until we have this information. The investigator(s) will discuss the results of these tests with you, and upon your request, will make copies of these results available to you. In the event that one of the test results is abnormal, you will be referred to your personal physician for follow-up.

In addition, you will be asked to complete the Global Physical Activity Questionnaire (GPAQ) to assess your typical physical activity habits. An interviewer will lead you through the questionnaire and record all responses. Further, you will be asked to record and submit a 3-day diet record prior to the study. This will be documentation of your usual daily diet. You will also be asked to complete a 1-day diet record during the baseline vascular testing visit to the lab. This will give us information about your sodium and potassium intake for that day which can be compared to the urine content of these nutrients. Your first controlled diet will begin the following day.

2. Assessment of Blood Vessel Function (not more than 8 visits to the lab; approx. 30 minutes-4 ½ hrs per visit, depending on the day, which is detailed below)

To determine the role of potassium on blood vessel function, we will have each participant go on a controlled potassium and sodium diet for 21 days. This is divided into 3 separate 7-day dietary regimens totaling 21 days.

During the strictly controlled potassium and sodium diet, all meals and snacks will be provided to you. Several days' worth of food will be packed in a cooler or in a bag. The food can be picked up at the University of Delaware STAR campus, 540 S. College Avenue, Newark, DE. A registered dietitian will prepare all food. All food provided should be consumed. If you choose not to eat some of the food, please return it so we can keep close track of exactly how much food you are eating. The total calories provided are designed to keep your weight stable during this period (this is referred to as "isocaloric"). We will also provide you with fluid (bottled water) to drink during this 21-day trial. This diet will contain 50% carbohydrates, 30% fat, and 20% protein. If you are on medications, you should continue to take these medications during this 21-day diet. Prior to starting the

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controlled potassium and sodium diet, we will confirm that all female subjects are not pregnant by performing an over-the-counter pregnancy test. To accomplish this, you will have to provide a small urine sample.

To establish the role of potassium on your blood vessel function, you will follow three one-week diet regimens at low and high amounts of potassium and sodium. On the low potassium/low sodium diet (7 days), you will consume approximately 1100 mg of potassium and 1100 mg of sodium each day. On the high potassium/high sodium diet (7 days), you will consume approximately 4700 mg of potassium and 7000 mg of sodium. For the low potassium/high sodium diet (7 days) you will consume approximately 1100 mg of potassium and 7000 mg of sodium. For reference, the CDC estimates that the average American consumes between 3,400 mg of sodium each day (this is equal to 1 1/2 teaspoons of table salt; table salt is sodium chloride). Therefore, the low sodium phase of this study contains far less sodium than most Americans consume, and the high sodium phase contains more sodium than the average American consumes. It is also estimated that the average adult consumes 2,400-3,300 mg of potassium and therefore you will consume a diet lower and higher than the average. The amount of sodium and potassium contained in the low and high phase of this dietary trial is consistent with the sodium and potassium content of many other national and international research studies.

During the baseline testing and dietary trial (over 21 days), you will be asked to come into the lab on days 7, 14, and 21 for the following (we request that you wear or bring athletic-type shorts to change into):

- Wear a blood pressure monitor on your upper arm for 24 hours
- Collect all of your urine into a container for 24 hours
- Assessment of blood vessel function
- Assessment of skin blood flow responses to local heating
- Have a standard intravenous catheter placed in your arm at the elbow crease for the collection of blood and cells from the vein wall
- Return of accelerometer for data retrieval and recharging.

Please note that during your baseline testing, you will not have the accelerometer. That will start with the dietary trial portion. Also, note that each of these visits during baseline and on days 7, 14, and 21 of the diet requires a post-lab visit to drop off equipment, so the total number of visits to the lab may be 8 during the study.

Accelerometer: You will be provided with an accelerometer (see photo at right) upon picking up food for the beginning of the 21-day diet. This is a small (1.8 in x 1.2 in) device that automatically measures your everyday daily living activity levels. We will ask that you wear this device on your hip at all times (except when bathing) over the 21-day diet. We also ask that you go about your normal, everyday activities as usual while wearing the device. You will be asked to return the device on the last post-lab visit (after day 21).

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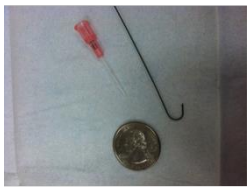

Blood Sample: About 5 tablespoons (2.5 fluid oz.) will be sampled from a catheter in a blood vessel in your arm, and some of the blood will be stored in a freezer for future analysis of hormones that control salt and water balance in the body. Your name will not appear on the stored blood sample; rather, the sample will be coded with a number. In addition, we will collect some cells from the wall of the vein. To do this, we will pass a very thin j-shaped wire through the intravenous catheter (see photo at right) and gently move it back and forth so it scrapes against the side of the vein wall. When this wire is moved against the vein wall, it will pick up some "endothelial" cells. We will then make measurements of different proteins in these cells that we collect.

24 hour blood pressure: On these same days, you will be asked to wear a blood pressure monitor on your upper arm for 24 hours. This blood pressure cuff will be set to automatically take your blood pressure every 20 minutes during the day. You will continue to wear the monitor at night while you sleep; it will automatically take your blood pressure every 30 minutes. If possible, you should sit down and relax when you feel the upper arm blood pressure start to inflate.

24 hour urine collection: During the same 24 hours you are wearing the blood pressure monitor on your arm, you will also be asked to collect all your urine into a plastic container. The urine sample will be analyzed for potassium and sodium content, as well as for the concentration of several hormones that control salt / water balance and blood pressure. Some of these analyses will be done immediately in the lab, and some of the urine sample will be stored in a freezer for future analysis. Your name will not appear on the stored urine sample; rather, the sample will be coded with a number.

Assessment of blood vessel function: This measurement consists of two parts. You will have three self-adhesive electrodes placed on your chest, and have to lie down for 15 minutes before starting the measurements.

- First, a pressure probe will be placed over the artery in your wrist on your skin and recordings of pressure taken. This will be followed by pressure recordings of the artery in your neck and groin. The pressure probe looks like a pen. A cloth tape measure will be used to measure the distance between the points on your neck and groin where these measurements were taken. We can make this measurement over your clothes.

- 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.

Assessment of skin blood flow responses to local heating on 4 days (baseline testing and the last day of each one week diet): Your skin makes natural substances when exposed to heat that cause the skin's blood vessels to get bigger which increases blood flow. We want to determine if the potassium and sodium content of the diet influences the skin blood flow response to heating. To do this we will use a technique referred to as "microdialysis." This

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technique involves placing very thin plastic tubing between the layers of your skin on your forearm. The tubing is about 6 times the diameter of a human hair. A small needle is used to place the tubing. The needle is removed as soon as the tubing is in place. This tubing allows us to pump small amounts of fluid directly into the skin. The substances that we pump through the tubing will leave the tubing and pass into the fluid in your skin. These substances (detailed below) are similar to some substances found naturally in your body and are known to influence skin blood flow responses. The substances infused into the tubing can only reach an area of the skin that is the size of a nickel. The following substances will be infused into the tubing:

1. Ringers solution (this is a salt-like solution)

2. Ringers solution plus L-NAME (this is a solution that blocks a natural substance in your body)

3. Ringers solution plus ascorbic acid (this is vitamin C)

4. Ringer's solution plus Tempol (an antioxidant that helps shut down cell reactions with free radicals)

5. Ringer's solution plus apocynin (this blocks an enzyme that produces free radicals)

We are using Tempol and apocynin in order to determine which free radicals (they are reactive molecules that can damage proteins and cell membranes as well other molecules in the body) may be increased as a result of alterations in dietary potassium and sodium. This will help us identify specific factors that increase free radicals with changes in dietary sodium and potassium. The skin blood flow will be assessed with a laser Doppler (light pointed toward the skin). We will heat the skin with a flat, nickel-sized probe attached to a heater that sits directly against the skin. The temperature of the heater can be very precisely controlled. We expect the skin blood vessels to dilate (get bigger) when we heat this small area of the skin. After we assess the blood flow responses to heating for 40 minutes, we will increase the temperature a few degrees and infuse a solution containing sodium nitroprusside, which is expected to cause the blood vessels to maximally dilate (get as large as possible).

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

There are no known risks associated with obtaining your height, weight, resting electrocardiogram, resting blood pressure, or providing a urine sample. You may have pain and/or bruising at the site where blood is taken or where the catheter is placed in your arm, and there is a small risk of infection. Fainting sometimes occurs during or shortly after blood is drawn. There may be minor discomfort associated with the placing and removing of electrodes. There are also no known risks in wearing the accelerometer.

There may be risks associated with repeated blood sampling. The risks associated with the use of the intravenous catheter include pain, inflammation of the vein, infection and blood clots, although the risks of blood clots and infection is very small. The risks associated with endothelial cell collection are similar to those of an intravenous catheter and include pain, inflammation of the vein, infection and blood clots, although the risks of blood clots and infection may be slightly greater (compared to intravenous placement alone) due to

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increased manipulation of the blood vessel. There is a low likelihood that the j-shaped wire could get stuck in the vein and/or be damaged; if this were to happen we would arrange additional follow-up medical care. If you require additional medical treatment, you will be responsible for the cost.

There are no known risks to providing a urine sample. There are no known risks to wearing a continuous blood pressure monitor on the upper arm. During waking hours, blood pressures are taken every 20-minutes, while at night they are taken every 30 minutes. You may experience minor discomfort when the cuff inflates, and it may wake you during sleep. There are minimal risks associated with the assessment of blood vessel function. You may feel some pressure or minor discomfort from the probes. You may feel minor discomfort when the blood pressure cuff is inflated, and may feel numbness and tingling in your arm and hand (similar to if your arm fell asleep).

There are minimal risks associated with consuming a low and high sodium diet. During the week you are consuming the low sodium diet, it is possible that you may occasionally feel lightheaded or dizzy upon standing. Alternately, during the high sodium diet, your blood pressure may rise to higher than normal levels. There could also be other symptoms that you experience during this 3-week diet. There is also a chance you may experience nauseousness and/or vomiting during the dietary sodium manipulation. We do not anticipate any discomfort with the low or high potassium diet. In the event that you experience symptoms that are bothersome, please inform the investigators. You are free to stop the diet anytime without penalty. We will keep track of your blood pressure by checking it every time you come into the lab.

There will be some minor discomfort associated with the placement of the tubing in the small area of forearm skin. We will place ice on your skin for 10 minutes to numb the area before we use the small needle to place the tubing in the skin. It is possible that you could have some redness and/or bruising at the site of the tube placement on your forearm. It is also possible that the tubing could break during removal from your skin in which case we will remove it by pulling it from the other end. If the tubing were to break on both ends during removal a small piece would be left under your skin. This small piece of tubing would be treated like a splinter. We are not aware of this occurring in any of the other laboratories that use this technique. Infection at the site of the tube placement is also possible. Sterile techniques and supplies like those used in a hospital minimize this risk. The substances (i.e. Ringer's solution, L-NAME, vitamin C, apocynin, and Tempol) that will flow through the tubing only go to a very small (nickel-sized) area of the skin. However, it is possible that you could have a bad reaction to one of these substances, which could cause redness, itching, rash, and/or local swelling. People allergic to Tylenol may be allergic to Tempol. Although unlikely, it is possible that a worse reaction could occur and cause fever, breathing problems, changes in heart rate, convulsions, and/or collapse. If a bad reaction should occur, medical help will be summoned immediately.

The "laser Doppler" used to measure skin blood flow will not damage your skin nor will it heat your skin. A separate heating source will be used to heat the skin. This will cause your skin to feel very warm but this will not hurt. The skin will turn red, just like it does when you take a hot bath. This redness will not last more than a few hours. If you feel that your arm is getting too hot you can tell us and we will reduce or stop the heating.

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There may be some discomfort associated with nitroglycerin administration. You may get a headache from the nitroglycerin. This is a very brief side effect that usually subsides within 10 minutes of administration. There is a risk that blood pressure will drop during nitroglycerin administration. This drop in blood pressure is also a brief side effect. Blood pressure values return to levels within 10 mmHg of baseline levels upon resolution of the testing. In the event that your blood pressure does not return to baseline, coupled with related symptoms, we will refer you for medical follow-up.

You should not participate in this study if you have donated blood, or had a large volume of blood drawn, within 4 weeks of study participation. In addition, you should not donate blood in the 4 weeks following study participation.

There are no known social, financial, or legal risks associated with participating in this study.

WHAT ARE THE POTENTIAL BENEFITS?

There may be no benefit to you for participating in this research study. The data collected during the screening session includes body composition assessment (i.e., percent body fat) and blood work. This information will be provided to you upon completion of the study.

HOW WILL CONFIDENTIALITY BE MAINTAINED?

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

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

Your research records may be viewed by the University of Delaware Institutional Review Board, but the confidentiality of your records will be protected to the extent permitted by law.

WILL THERE BE ANY COSTS RELATED TO THE RESEARCH?

There are no costs associated with participating in the study.

WILL THERE BE ANY COMPENSATION FOR PARTICIPATION?

All subjects will receive $350.00 for full completion of the baseline testing and 21-day dietary trial (phases #1 and #2, the Screening and Assessment of Blood Vessel Function) to offset the cost of transportation, etc. Those who complete only the baseline testing will receive $50; those who complete only through the 1-week of the dietary trial, but not the second or third week of the dietary trial, will receive $150.00; and those who complete

Participant Initials _____

through week 2 of the dietary trial will receive $250. Those who only complete phase #1 (the screening session) will not be compensated.

WHAT IF YOU ARE INJURED BECAUSE OF THE STUDY

If you are injured during research procedures, you will be offered first aid at no cost. If you need additional medical treatment, the cost of this treatment will be your responsibility or that of your third-party payer (for example, your health insurance). By signing this document, you are not waiving any rights that you may have if injury was the result of negligence of the university or its investigators.

DO YOU HAVE TO TAKE PART IN THIS STUDY?

Taking part in this research study is entirely voluntary. You do not have to participate in this research. If you choose to take part, you have the right to stop at any time. If you decide not to participate or if you decide to stop taking part in the research at a later date, there will be no penalty or loss of benefits to which you are otherwise entitled. Your refusal will not influence current or future relationships with the University of Delaware.

WHO SHOULD YOU CALL IF YOU HAVE QUESTIONS OR CONCERNS?

If you have any questions about this study, please contact the Principal Investigator, Shannon Lennon-Edwards at 302-831-2798.

If you have any questions or concerns about your rights as a research participant, you may contact the University of Delaware Institutional Review Board at 302-831-2137.

Your signature on this form means that: 1) you are at least 18 years old; 2) you have read and understand the information given in this form; 3) you have asked any questions you have about the research and those questions have been answered to your satisfaction; 4) you accept the terms in the form and volunteer to participate in the study. You will be given a copy of this form to keep.

Participant Signature _____ Date: _____

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

FUTURE STUDIES

Do we have your permission to contact you regarding participation in future studies? Please indicate this by placing a check mark in the box below and initialing it.

YES: ☐ NO: ☐ Participant Initials/Date:_____

Appendix B

ONE DAY DIET RECORD

INSTRUCTIONS FOR KEEPING YOUR ONE DAY DIET RECORD

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

I. Details on recording food intake:

1. ***Only record on day of first (habitual) visit.***
2. Use the provided food log sheets **to write down everything you eat and drink.** Please avoid holidays, birthdays, party days, or any day that is out of the ordinary.
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.
8. If you eat in a fast food restaurant, **write down the place as well as the foods you ate,** as specific brand names can help us in our analysis of your diet.

Estimating Portion sizes

Fruits and Vegetables

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

Meats, nuts, and other protein rich foods

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

Dairy

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

Breads and grains

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

Fats

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

Desserts

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

FOOD INTAKE LOG

Day of the Week:_____

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

Appendix C

ADDITIONAL DIETARY DATA

Table C.1 3-day diet record

Variables	Subjects (N=9)
Energy (kcal)	2121 ± 221
Total Carbohydrates (g)	249 ± 32
Total Protein (g)	79 ± 8
Total Fat (g)	85 ± 11
Carbohydrate (%)	46 ± 3
Protein (%)	15 ± 1
Fat (%)	35 ± 2
Cholesterol (mg)	321 ± 62
Saturated Fat (g)	30 ± 5
Monounsaturated fat (g)	28 ± 4
Poly-saturated Fat (g)	20 ± 3
Sodium (mg)	3667 ± 450
Potassium (mg)	2422 ± 112
Chloride (mg)	967 ± 129
Phosphorous (mg)	1279 ± 116
Iron (mg)	18 ± 2
Zinc (mg)	10 ± 1
Copper (mg)	1.29 ± 0
Manganese (mg)	3.94 ± 0
Selenium (mg)	114.17 ± 10
Magnesium (mg)	309.14 ± 21
Vitamin D (calcifierol) (mcg)	4. ± 0.7
Vitamin A (Retinol Equivalents) (mcg)	1090 ± 178
Vitamin Beta-carotene (mcg)	3810 ± 1281
Vitamin E (Total alpha-tocopherol) (mg)	11 ± 1
Vitamin K (mcg)	114 ± 19
Vitamin C (mg)	82 ± 18
Vitamin B1 (mg)	1 ± 0.1
Vitamin B2 (mg)	1 ± 0.2
Vitamin B3 (mg)	25 ± 3
Pantothenic acid (mg)	5 ± 0.4
Vitamin B6 (mg)	2 ± 0.2
Folate (mcg)	559 ± 97

Vitamin B12 (mcg)	3 ± 0.5
Alcohol (g)	11 ± 4
Caffeine (mg)	74 ± 18
Water (g)	1742 ± 307

Table C.2 24-hour diet record

Variables	Subjects (N=5)
Energy (kcal)	2246 ± 232
Total Carbohydrates (g)	258 ± 22
Total Protein (g)	102 ± 21
Total Fat (g)	94 ± 18
Carbohydrate (%)	46 ± 5
Protein (%)	17 ± 2
Fat (%)	35 ± 4
Cholesterol (mg)	466 ± 131
Saturated Fat (g)	34 ± 7
Monounsaturated fat (g)	32 ± 7
Poly-saturated Fat (g)	20 ± 4
Sodium (mg)	4279 ± 813
Potassium (mg)	2645 ± 489
Chloride (mg)	1358 ± 274
Phosphorous (mg)	1623 ± 231
Iron (mg)	20 ± 7
Zinc (mg)	10 ± 1
Copper (mg)	1 ± 0.1
Manganese (mg)	4 ± 1
Selenium (mg)	157 ± 32
Magnesium (mg)	339 ± 58
Vitamin D (calcifierol) (mcg)	7 ± 1
Vitamin A (Retinol Equivalents) (mcg)	1549 ± 387
Vitamin Beta-carotene (mcg)	4362 ± 2669
Vitamin E (Total alpha-tocopherol) (mg)	18 ± 10
Vitamin K (mcg)	114 ± 66
Vitamin C (mg)	193 ± 72
Vitamin B1 (mg)	1 ± 0.2
Vitamin B2 (mg)	1 ± 0.1
Vitamin B3 (mg)	28 ± 6
Pantothenic acid (mg)	5 ± 0.1
Vitamin B6 (mg)	2 ± 0.3
Folate (mcg)	526 ± 106
Vitamin B12 (mcg)	3 ± 0.5
Alcohol (g)	.2 ± 0.2
Caffeine (mg)	46 ± 46

Water (g)	2212 ± 304