

**THE EFFECT OF DIETARY COUNSELING ON VASCULAR
FUNCTION IN HIGH SODIUM CONSUMERS: A PILOT STUDY**

by

Karen A. Solecki

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the Master of Science in Human Nutrition

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Karen A. Solecki

Approved: _____
Shannon Lennon-Edwards, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____
Michael Peterson, Ed.D
Chair of the Department of Behavioral Health and Nutrition

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

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

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ABSTRACT

Cardiovascular disease (CVD) is the leading cause of death in the United States for both men and women. Research has shown that a low sodium diet is beneficial for cardiovascular health including blood vessel health. Dysfunction of the blood vessels, or endothelial dysfunction, can lead to atherosclerosis and heart disease. High sodium intakes have been shown to diminish endothelial function while low sodium intakes have been associated with improved endothelial function. To date, much of this research has been done in a controlled feeding environment. While dietary counseling is a method that has been used to lower sodium intake to improve blood pressure, this approach has not been utilized to assess the impact of a low sodium diet on endothelial function in habitually high salt consumers who are free of hypertension and obesity. Therefore, the purpose of this study was to determine the effect of following a low sodium diet (2g/day) for 30 days on endothelial function in high sodium consumers (≥ 5 g/day) independent of changes in blood pressure. Vascular function tests at baseline and 30 days were performed to determine brachial artery flow-mediated dilation, microvascular function, augmentation index (AIx) as assessed by pulse wave analysis, and carotid-to-femoral pulse wave velocity (PWV). Subjects underwent 24-hour blood pressure monitoring and collection of a 24-hour urine at baseline, midpoint, and 30 days. Subjects were counseled by a Registered Dietitian to achieve the 2g-sodium diet, and followed weekly to encourage compliance. Eight habitually high sodium consumers (6 M/2 F; age 35.3 ± 5.6 yrs) completed the dietary intervention study. All subjects were able to successfully lower

their sodium intake from an average at baseline of 5284 ± 387 mg to an average of 1778 ± 409 mg at the fifth dietary recall ($p < 0.05$), with no changes in blood pressure ($p > 0.05$). This was confirmed by a non-significant decrease in urinary sodium excretion at day 30. Brachial artery FMD at baseline ($7.29 \pm 1.22\%$) and at day 30 ($7.80 \pm 1.5\%$) did not significantly differ over the 30-day period. When separating males from females, there was a trend toward significance between baseline and day 30 ($p = 0.13$). Carotid-to-femoral PWV and AIx did not significantly differ over the thirty-day period. We did not see any significant changes in the nitric oxide mediated Plateau phase at the Ringer's site over the study intervention, or between the Ascorbic Acid site and Ringers, suggesting no improvements in microvascular function. In conclusion, dietary counseling to lower sodium intake in habitually high sodium consumers for 30 days resulted in lower dietary sodium intake levels, but did not significantly affect vascular function.

Chapter 1

BACKGROUND AND SIGNIFICANCE

1.1 Introduction to Cardiovascular Disease

Cardiovascular disease (CVD) is the leading cause of death for both men and women in the United States. According to the most recent statistics from the American Heart Association (AHA), an estimated 83.6 million American adults (>1 in 3) currently have CVD. Over 600,000 people die of CVD every year; that is about 1 in every 4 deaths (Kochanek, Xu, Murphy, Minino, & Kung, 2011). The total direct and indirect cost of CVD and stroke in the US for 2010 was estimated to be \$315.4 billion (Go et al., 2014). Risk factors for CVD include high blood pressure, having an abnormal lipid profile, diabetes mellitus, physical inactivity, tobacco use, consuming a poor diet, and overweight/obesity. With the increased cost of treating CVD, and the number of individuals with multiple risk factors on the rise, it is prudent to apply intervention strategies to prevent the development of CVD and related mortality.

Studies have shown that high sodium intake correlates with cardiovascular mortality (Umesawa et al., 2008), high blood pressure (Drenjancevic-Peric et al., 2011; INTERSALT, 1988; Todd et al., 2010), and endothelial dysfunction (Dickinson, Clifton, & Keogh, 2011; DuPont et al., 2013), while sodium reductions have been shown to decrease blood pressure (Hodson, Harnden, Roberts, Dennis, & Frayn, 2010; Pimenta et al., 2009; Sacks et al., 2001; Svetkey et al., 2004) and improve endothelial dysfunction (Dickinson, Keogh, & Clifton, 2009; Dickinson, Clifton, & Keogh, 2014b; Jablonski et al., 2013b). Dysfunction of the blood vessels or endothelial

dysfunction precedes the development of atherosclerosis, which is a broad term that describes the thickening of blood vessel walls and their subsequent loss of vascular elasticity. Atherosclerosis plays a large role in the development of coronary artery disease (CAD) and stroke, which are two types of CVD with high mortality rates. Focus on attaining healthy endothelial function is thus important to improve overall cardiovascular health.

1.2 The Endothelium

The rise in CVD can be attributed to the aforementioned risk factors as well as endothelial dysfunction, which can precede the development of atherosclerosis (Anderson et al., 1995; Shimokawa, 1999). The endothelium is a single layer of endothelial cells lining the lumen of the blood vessel with the smooth muscle cells underneath. The primary function of the endothelium is to maintain vascular homeostasis in the body (Widlansky, Gokce, Keaney, & Vita, 2003), which occurs when endothelial cells release vasoactive substances (Amezcuca, Dusting, Palmer, & Moncada, 1988; Ray & Shah, 2005) in response to hemodynamic forces (Verma & Anderson, 2002). These vasoactive substances include the vasodilators nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF), prostacyclin (PGI₂) and the vasoconstrictors endothelin-1 (ET-1) and angiotensin II. In general, these vasoactive substances regulate vascular tone, control the vascular inflammatory process by producing cytokines and adhesion molecules, and are capable of balancing blood fluidity and coagulation (Widlansky et al., 2003).

Nitric oxide is the key relaxing factor of the endothelium, and acts as an agonist to the contracting substances ET-1 and angiotensin II, thus regulating vascular reactivity (Calver, Collier, & Vallance, 1993; Furchgott & Zawadzki, 1980). Nitric

oxide and L-citrulline are produced from L-arginine in the presence of the enzyme nitric oxide synthase (NOS) (Roe & Ren, 2012; Verma & Anderson, 2002). There are three isoforms of NOS: iNOS, nNOS, and eNOS that can facilitate this conversion. Endothelial nitric oxide synthase (eNOS) is the enzyme of interest, as it produces NO that acts in the endothelium (Barouch et al., 2002; Calver et al., 1993; Verma et al., 2002). In this reaction, eNOS requires cofactors such as tetrahydrobiopterin (BH₄), nicotine amide dinucleotide phosphate (NADPH), flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and calmodulin (Calver et al., 1993; Ignarro, 1996; Verma et al., 2002). After NO is formed in the endothelium, it diffuses into the underlying vascular smooth muscle cell resulting in a fall in the intracellular calcium levels leading to smooth muscle relaxation (i.e. vasodilation) (Calver et al., 1993; Myers, Minor, Guerra, Bates, & Harrison, 1990). This is an important physiologic process, since vasodilation will allow more blood flow through the body and to target organs.

Nitric oxide's action can be enhanced or inhibited by a variety of molecules. Acetylcholine (ACh) has been shown to produce vasodilatory effects on the endothelium (Furchgott & Zawadzki, 1980) while asymmetric dimethyl arginine (ADMA) acts to inhibit the enzyme eNOS, which will lead to an increase in vascular tone, blood pressure, and reduced forearm blood flow (Vallance, Leone, Calver, Collier, & Moncada, 1992). Impairment in the dilation of the blood vessels can lead to decreased coronary blood flow, resulting in decreased myocardial perfusion and myocardial ischemia. This dysfunction of the blood vessels can ultimately result in heart disease (Thijssen et al., 2011).

1.2.1 Endothelial Dysfunction and Atherosclerosis

Endothelial dysfunction can be generally described as decreased production of or availability of NO, or a disproportion in the relative contribution of endothelium-derived relaxing and contracting factors (Verma & Anderson, 2002). Nitric oxide plays a key role in inhibiting processes contributing to the development of atherosclerosis (Verma & Anderson, 2002). These processes include inhibition of smooth muscle proliferation and migration, oxidation of LDL, platelet aggregation, monocyte adhesion, and synthesis of inflammatory cytokines. Nitric oxide will also oppose the actions of vasoconstrictors (Verma & Anderson, 2002). A damaged endothelium will exhibit increased expression of adhesion molecules to the endothelial cells, which leads to a decrease in their ability to release vasoactive substances (Verma & Anderson, 2002). With a decreased release of NO, the anti-atherosclerotic actions will be diminished as well. Hence, any disruption in NO availability or production not only leads to a loss of dilation, but atherosclerotic progression.

During atherosclerosis, endothelium dependent relaxation may be reduced from alterations in endothelial signal transduction, limited L-arginine availability, limited cofactor availability, altered eNOS expression, the presence of reactive oxygen species (ROS), intimal thickening as a diffusion barrier, impaired smooth muscle response to NO, or production of endothelial derived contracting factors (Shimokawa, 1999). Increased levels of oxidative stress from ROS can lead to a pro-inflammatory state, a reduced vasodilation, and enhanced blood clot formation (Endemann & Schiffrin, 2004) leading to endothelial dysfunction. Oxidative stress occurs when there is an imbalance between pro-oxidants (superoxide $O_2^{\cdot-}$; hydroxyl radical OH^{\cdot} ; hydrogen peroxide H_2O_2 ; peroxynitrite $ONOO^{\cdot}$) and antioxidants

(ascorbic acid; catalase; superoxide dismutase SOD) in the body, usually with an excess of pro-oxidants (Manning, Meng, & Tian, 2003).

Superoxide has the capability of scavenging NO to form ONOO⁻, therefore decreasing the amount of NO and leading to an increase in oxidants (Rubanyi & Vanhoutte, 1986; Tschudi & Luscher, 1996). ONOO⁻ will then oxidize a critical cofactor in the eNOS pathway, BH₄ (Tiefenbacher, 2001). A deficiency of BH₄ will lead to an uncoupling of eNOS, resulting in the creation of more O₂⁻ and H₂O₂ instead of NO. This state of oxidative stress leads to a decrease in the bioavailability of NO due to its' altered production (Roe & Ren, 2012; Verma & Anderson, 2002).

1.2.2 Assessment of Vascular Health

Assessment of the endothelium is important as it plays a large role in maintaining vascular homeostasis in the body (Widlansky et al., 2003). There are a variety of characteristics, such as aging, menopause (Shimokawa, 1999), smoking (Messner & Bernhard, 2014) and a high salt intake (Dickinson et al., 2009; DuPont et al., 2013) that are associated with endothelial dysfunction. Brachial artery flow-mediated dilation (FMD) is a technique that measures endothelial-dependent dilation. Measurement of the peripheral conduit brachial artery in humans using FMD corresponds well to the coronary artery vasodilatory function (Anderson et al., 1995). In this study, vasodilation in the coronary artery induced from administration of acetylcholine was similar to the dilation produced from the shear stress produced during assessment of FMD (Anderson et al., 1995). This technique provokes the release of NO from the endothelium in response to the shear of blood flow, resulting in vasodilation (Corretti et al., 2002). Impaired FMD is considered an early marker of CVD (Go et al., 2014). The Multi-Ethnic Study of Atherosclerosis (MESA) measured

FMD during an initial examination period in the year 2000 in over 3,000 older (average age 61 years) individuals free of CVD. Participants were followed for 5 years. FMD was found to be inversely associated with CV events, even after adjusting for a variety of factors (age, sex, diabetes, smoking, LDL-C, medications, etc.). The authors reported that an increased FMD (indicative of better brachial function) resulted in a 16% decreased risk of CVD development (Yeboah et al., 2009). This study demonstrated that assessment of FMD might predict CVD development in both adult men and women from a wide range of ethnic backgrounds.

1.3 Nutrition and Cardiovascular Health

The AHA sets major goals each decade for the reduction of CVD in the U.S. Of the 2010 goals, only a few were achieved. Therefore, one new goal for 2020 aims to improve the CV health of all Americans by 20%, to reduce deaths from all CVD's and in particular, deaths from stroke by 20%. The AHA has identified seven CV health metrics that are important to determining an individuals' CV health. These metrics include smoking, physical inactivity, poor diet, energy balance and weight, high blood pressure, high cholesterol, and an elevated fasting plasma glucose level. *Ideal cardiovascular health*, as defined by the AHA, is the absence of clinically manifest CVD together with the simultaneous presence of optimal levels of all 7 metrics. The major goals of these health metrics are to promote both healthy behaviors (healthy diet patterns, appropriate energy intake, physical activity, and nonsmoking) and healthy biomarker levels (optimal blood lipids, blood pressure, glucose levels) throughout the lifespan (Go et al., 2014).

The range of US adults meeting all 7 health metrics and qualifying for an *ideal cardiovascular health* profile is currently low, and ranged from 0.0-0.3% in 2009-

2010. In 2009-2010, less than 1% of Americans met at least 4 of 5 healthy dietary goals. This demonstrates that dietary metric goals are one of the lowest category percentages that Americans have achieved. In particular, there were only 0.6% of Americans consuming less than 1,500 mg/day of sodium in 2009-2010, one of the dietary metric goals (Go et al., 2014).

1.3.1 Sodium Guidelines

The 2010 U.S. Dietary Guidelines for Americans recommends a daily sodium intake of less than 2,300 mg, with a further recommendation of less than 1,500 mg for certain populations (USDA & USDHHS, 2010). These populations include African Americans, individuals with hypertension, diabetes mellitus, chronic kidney disease, or over the age of 51 years. The estimated average intake of sodium in the US population over 2 years of age is 3,400 mg/day (USDA & USDHHS, 2010). The Adequate Intake (AI) for sodium is 1,500 mg/day recommended by the Institute of Medicine (IOM), and a tolerable upper intake level (UL) of 2,300 mg/day. The AHA currently recommends consuming <1,500 mg sodium per day. Data from the NHANES studies illustrates that the 88.2% of US population is consuming in excess double the amount of the recommended 2,300mg daily (Centers for Disease Control and Prevention (CDC), 2011).

1.3.2 Sources of Salt/Sodium in the Diet

The majority of sodium consumed in the diet is in the form of salt. However, table salt (i.e. salt added during cooking and at the table) contributes only a small portion of the sodium consumed. Most salt consumed by Americans is found in processed and ready to eat foods. A study by Drewnowski et al. (2013) examined food

sources and location of sodium consumption using NHANES (2003-2008) data in over 22,000 individuals. They found that the majority of sodium consumed in the US came from foods bought and already prepared. While stores provided the majority of sodium in the diet (58.1%-65.2%), full service restaurants and quick service restaurants (take-out or delivery) provided a slightly smaller portion (18.9%-31.8%), followed by schools, the workplace, and vending machines. Foods providing the majority of dietary sodium consumed included pizza, yeast breads, and chicken mixed dishes.

1.4 The Effect of Sodium on the Vasculature

Excess sodium intake is detrimental to the cardiovascular system (O'Donnell et al., 2015). Sodium is an important electrolyte with many functions, including its role in proper nerve and muscle function, as well as controlling extracellular fluid volume (ECV) (de Wardener, He, & MacGregor, 2004). Extracellular fluid volume is important because it is essential for maintaining the balance of fluid in our body. The body balances the levels of sodium and fluid volume through how much we ingest as well as how much we excrete through the kidneys. When we ingest excess amounts of sodium, the kidneys are presented with the obstacle of handling and excreting this. Too much sodium can also negatively affect the cardiovascular system, and can predispose individuals to high blood pressure (Ha, 2014).

Too much salt in the body can lead to high blood pressure as excess sodium increases fluid volume. This increased fluid volume traveling through the blood vessels over time will lead to increased vessel wall thickening and reduced vessel diameter, making blood flow through these vessels difficult. Now, the heart will have to work harder and pump more blood so it can flow through the narrow blood vessels.

The kidneys also play a role in blood pressure regulation through the renin-angiotensin-aldosterone system (RAAS) (Drenjancevic-Peric et al., 2011). The RAAS helps to regulate arterial pressure and ECF volume by releasing various enzymes and substances to induce constriction and relaxation. In the case of low blood volume or low ECF, sensor cells known as baroreceptors in the blood vessels cause release of the hormone renin from the kidneys. Renin helps to convert angiotensinogen (which is produced from the liver) to angiotensin I, a vasoconstrictor peptide (Drenjancevic-Peric et al., 2011). Angiotensin I is then converted to angiotensin II by angiotensin converting enzyme (ACE), an enzyme that is secreted from the endothelium of lung vessels. Essential hypertension has been treated successfully using medications that act to inhibit ACE, as well as blocking angiotensin II receptors, thus limiting the production of this vasoconstrictor. This demonstrates that the RAAS plays a role in the pathophysiology of hypertension (Drenjancevic-Peric et al., 2011).

Angiotensin II can elevate arterial pressure and its' responsiveness depends on two receptors, AT₁ and AT₂ receptors. The AT₁ receptor mediates its vasoconstrictor capabilities, leading to an increase in total peripheral resistance, and a subsequent rise in arterial pressure. Angiotensin II can decrease salt and water excretion by the kidneys through the stimulation of the hormone aldosterone from the adrenal cortex, release of vasopressin, and an increase in thirst in order to increase blood volume (Crawley et al., 2006 & Phillips 1987). When the sodium levels increase, there will be an increase in osmotic pressure pulling fluid into the blood leading to a normal blood volume. Vasopressin will lead to an increase in blood pressure by causing water reabsorption in the kidneys (Phillips, 1987). This can lead to an increase in blood volume, which means a greater volume traveling through the blood vessels. This high

volume will lead to the widening of the vessels, which will build up with muscle over time in order to support the increased volume. This results in a smaller vessel diameter, meaning a slower blood flow and less nutrients and oxygen traveling through the body.

Angiotensin II has also been thought to play a role in endothelial dysfunction through directly acting on the endothelium as a vasoconstrictor as well as increasing oxidative stress. Nitric oxide and angiotensin II work against one another to maintain vascular and renal homeostasis in the body (Zhou, Adam, Jaimes, & Raij, 2003). Nitric oxide will antagonize the vasoconstrictive and pro-atherosclerotic effects (thrombotic, inflammatory, fibrotic effects on the endothelium) of angiotensin II, whereas angiotensin II decreases NO bioavailability by promoting oxidative stress. Zhou et al., (2003) demonstrated that a high salt diet increased production of $O_2^{\cdot -}$ through upregulation of angiotensin II in Dahl Salt Sensitive rats. Increased levels of $O_2^{\cdot -}$ can lead to a decrease in the availability of NO resulting in impaired vasodilation and ultimately endothelial dysfunction.

Knape et al., (1988) demonstrated that a high salt diet leads to increased sensitivity of angiotensin II to the vascular angiotensin II receptors, leading to an increase in constriction. In some cases, a dietary sodium restriction in humans has been shown to increase plasma aldosterone and angiotensin II levels (Brown et al., 1972). Angiotensin II and NO are both synthesized by the endothelial cells and released for their respective actions. The cycle of vasoconstriction will continue if levels of angiotensin II continue to be increased resulting in stimulation of the endothelial cells to synthesize and release another potent vasoconstrictor, endothelin-1 (Sasser, Pollock, & Pollock, 2002; Schulman, Zhou, & Raij, 2006).

1.4.1 Dietary Sodium and Endothelial Dysfunction

Much focus has been made on the role of dietary sodium on blood pressure and consequently CVD development. Indeed, many national agencies including the Academy of Nutrition and Dietetics, the AHA, the Centers for Disease Control and Prevention, the US Department of Agriculture and US Department of Health and Human Services, as well as the World Health Organization (WHO) recommend lowering sodium intake as a way to decrease blood pressure and to prevent CVD and stroke (Whelton et al., 2012). This is based on studies that have demonstrated a reduction in blood pressure in response to a sodium reduction (Aburto et al., 2013; Forte, Miguel, Miguel, de Padua, & Rose, 1989; He, Li, & Macgregor, 2013). However, more recent research has shown that dietary sodium can be detrimental to endothelial function independent of blood pressure (Dickinson et al., 2011; Dickinson et al., 2014b; DuPont et al., 2013). This suggests that excess sodium has a direct, negative effect on the functions of the endothelium without any changes seen in blood pressure. Endothelial dysfunction is thought to precede the development of atherosclerosis, which can ultimately lead to CVD and mortality (Shimokawa, 1999).

A cross-sectional study by Jablonski et al., (2009) demonstrated that lower self-reported sodium intakes were associated with enhanced brachial artery FMD as opposed to higher sodium intakes. Subjects who consumed less than 100 mmol/day were found to have a 52% greater FMD compared to those who consumed between 100-200 mmol/day. Similar findings have been found in other studies that restricted dietary sodium intake and saw improved endothelial function (Dickinson et al., 2009; DuPont et al., 2013; Jablonski et al., 2013b).

Studies have looked at both the acute and chronic effects of sodium intake on endothelial function. Acutely, a high salt meal (HSM) impaired FMD compared to a low salt meal (LSM) in normotensive middle-aged healthy adults (Dickinson et al., 2011). This impairment was seen at 30-mins post-ingestion and was sustained at 120-minutes. The HSM included 65mmol of Na⁺ (1,380 mg), which is about 92% of the daily intake of sodium recommended by the AHA, while the LSM contained 5 mmol Na⁺ (115mg). Further, a dietary sodium reduction of 3 grams/day improved brachial artery FMD after only 2 days and was sustained through 6 weeks in overweight and obese adults (Dickinson et al., 2014b). These studies suggest that changes to endothelial function can occur quickly and be sustained if dietary sodium restrictions are maintained.

Several studies have been conducted that looked at longer or chronic interventions. Lowering sodium intake from 150 mmol/day to 50 mmol/day produced improvements in FMD after 2 weeks in overweight/obese normotensive adults independently of blood pressure (Dickinson et al., 2009). Further, four weeks of dietary sodium restriction improved endothelial function in a middle-aged population with mildly elevated SBP when intakes of 1200-1500mg were achieved compared to a normal sodium diet of 3,600 mg/day (Jablonski et al., 2013b). While both studies showed improvements in endothelial function, subjects were consuming diets that are below the sodium recommendations.

Additionally, endothelial function has been altered after 7 days of dietary sodium loading in a controlled feeding study (DuPont et al., 2013). DuPont et al., (2013) demonstrated that a dietary sodium intake of 300-350 mmol/day compared to a low sodium intake of 20 mmol/day impairs brachial artery FMD independently of

blood pressure. Further, impairments in arterial vascular tone after dietary salt loading have been seen in hypertensive adults (Todd et al., 2010). Aortic pulse wave velocity (PWV), a measure of aortic stiffness, was significantly increased after 4 weeks of consuming 150-200 mmol Na/day compared to 60 mmol/day. Hence, high sodium intakes not only impair endothelial function, but also can lead to stiffening of the large arteries.

1.4.1.1 Potential Mechanisms

Sodium is thought to be detrimental to vascular health by several mechanisms. One mechanism is through the increased production of ROS. As stated earlier, the superoxide anion $O_2^{\cdot-}$, the hydroxyl radical OH^{\cdot} , hydrogen peroxide H_2O_2 , and peroxynitrite $ONOO^-$ are pro-oxidants that, when produced in excess of antioxidants (ascorbic acid; catalase; superoxide dismutase, SOD) can cause damage to the endothelium (Manning et al., 2003). This state of imbalance can lead to endothelial dysfunction when there is a decrease in the bioavailability of NO due to its' altered production (Roe & Ren, 2012; Verma & Anderson, 2002).

Several animal model studies have demonstrated the link between a high salt diet and increased levels of oxidative stress. A high salt diet has been shown to decrease arteriolar vasodilation in response to endogenous acetylcholine in a rodent model (Lenda, Sauls, & Boegehold, 2000). When SOD + catalase as well as TEMPOL (an SOD mimetic) + catalase were infused, dilatory responses were restored. This demonstrated that generation of ROS decreased NO bioavailability, as responses to the NO-donor sodium nitroprusside (SNP) were similar between the high salt and low salt diet. Additionally, a high salt diet (7%) decreased vasodilation in the

spinotrapezius muscle arterioles of mice and this was attributed to increased $O_2^{\cdot-}$ generation (Nurkiewicz & Boegehold, 2007).

High salt diets have also been shown to reduce NO levels by decreasing NOS activity (Banday, Muhammad, Fazili, & Lokhandwala, 2007). Salt can disrupt the production of NO from L-arginine by inactivating the enzyme eNOS (Li et al., 2009). Schlaich et al., (2004) found that altered L-arginine transport may play a role in limiting NO bioavailability, particularly in hypertensive individuals and those that are genetically predisposed to hypertension. Supplementation with L-arginine was found to improve endothelial dependent dilation (Schlaich et al., 2004).

A role for sodium-induced oxidative stress levels in the vasculature has been shown in a human model as well. Infusion of ascorbic acid preserved NO-mediated vasodilation in the microvasculature under high sodium conditions in a group of normotensive adults (Greaney et al., 2012). Ascorbic acid is a non-specific scavenger of $O_2^{\cdot-}$ radicals and therefore, further investigations are needed with additional antioxidants.

1.4.2 Dietary Sodium and Microvascular Function

While many studies have evaluated a larger conduit vessel such as the brachial artery, the microvasculature has been shown to be impaired in response to excess sodium (DuPont, Farquhar, & Edwards, 2011; Greaney et al., 2012). The microvasculature includes the smallest systems of blood vessels in the body, which are responsible for microcirculation (distributing blood to the tissues); included are arterioles, capillaries, metarterioles and venules. The cutaneous circulation is an accessible and potentially representative vascular bed that allows one to look at the underlying mechanisms and processes of microcirculation. Vascular dysfunction can

be seen in the cutaneous circulation, and may reflect general systemic vascular dysfunction. Alterations in microvascular function may occur in the early stages of CVD (DuPont et al., 2011; Holowatz, Thompson-Torgerson, & Kenney, 2008).

Infusion of a hypertonic saline into the cutaneous circulation containing a 3% NaCl solution resulted in significant alterations of skin blood flow (DuPont et al., 2011) and demonstrated a reduction in NO production suggesting that excess sodium affects the smallest blood vessels in our body. This response has also been seen after 7 days of a high sodium diet (350 mmol/day) in salt resistant individuals (Greaney et al., 2012). Salt-resistance was defined as < 5 mmHg change in MAP from a low to high sodium diet. As mentioned previously, this study demonstrated that oxidative stress plays a role in the diminished function of the microvasculature as ascorbic acid infusion improved vasodilation.

1.4.3 Dietary Sodium and Arterial Stiffness

Pulse Wave Velocity (PWV) evaluates arterial distensibility and has been found to predict future CV events. It is considered an independent predictor of all-cause mortality and CV mortality (Laurent et al., 2001). Pulse wave velocity increases with stiffer arteries, and a high PWV translates into a less elastic vessel seen along the aorta and aortoiliac pathway (Laurent et al., 2001). In patients without any atherosclerotic alterations, CV risks are increased with an increasing PWV. A PWV of >13 m/s by itself has been shown to be a strong predictor of CV mortality (Blacher, Asmar, Djane, London, & Safar, 1999). However, PWV and CV risk are much higher in those with established atherosclerotic alterations (Blacher et al., 1999). This demonstrates that aortic PWV is strongly associated with the presence and

development of atherosclerosis and should be used as a marker of CV risk and disease in hypertensive patients (Blacher et al., 1999).

A high sodium diet can cause arteries to stiffen. Dietary sodium loading studies have shown increases in PWV and blood pressure after a period of 4 weeks (Todd et al., 2010). Consumption of a high salt meal has also been shown to increase augmentation index (AI) immediately following in healthy, normotensive adults (Dickinson, Clifton, Burrell, Barrett, & Keogh, 2014a).

1.5 Dietary Counseling

Dietary counseling is a useful tool that has been used to facilitate dietary changes in individuals (Blumenthal et al., 2010; Cakir & Pinar, 2006; Dickinson et al., 2009; Dickinson et al., 2014b; Hodson et al., 2010; Jablonski et al., 2013b; Osterdahl, Kocturk, Koochek, & Wandell, 2008; Todd et al., 2010). Time frames for counseling interventions have ranged from 2 weeks (Dickinson et al., 2009) to 6 months (Blumenthal et al., 2010; Cakir & Pinar, 2006) with various intervention periods in between (Dickinson et al., 2014b; Hodson et al., 2010; McMahon et al., 2013; Todd et al., 2010). Specifically, dietary counseling focused on changing sodium intake have shown improvements in blood pressure in normotensive (Hodson et al., 2010; Sacks et al., 2001; Svetkey et al., 2004), prehypertensive (Svetkey et al., 2004), and hypertensive individuals (Cakir & Pinar, 2006; Sacks et al., 2001; Svetkey et al., 2004). Dietary counseling has also been shown to improve endothelial function in normotensive (Dickinson et al., 2009; Dickinson et al., 2014b) and prehypertensive individuals (Jablonski et al., 2013b). While timeframes have varied, a period of 30-days has been shown to be an effective length of time to see decreases in blood

pressure while maintaining 94%-100% of their original participants at the end of the study (Hodson et al., 2010; Sacks et al., 2001; Svetkey et al., 2004).

Improvements in endothelial function assessed via FMD due to a dietary sodium reduction have been demonstrated in counseling studies with overweight/obese normotensive (Dickinson et al., 2009; Dickinson et al., 2014b) and pre-hypertensive individuals' (Dickinson et al., 2009; Dickinson et al., 2014b; Jablonski et al., 2013b). Dietary counseling can be defined as the process of guiding a person toward a healthy nutrition lifestyle by meeting nutritional needs and solving problems that are barriers to change. In counseling studies, nutrition professionals including a registered dietitian, are utilized to aid subject compliance and provide all nutrition education in order to achieve the desired dietary sodium levels. Counseling studies are of interest because of their generalizability to the worldwide population.

Improvements in endothelial function have been seen quickly in some cases. Two days of consuming a low salt diet achieved with a combination of dietary counseling provided by a Registered Dietitian (RD), as well as provision of some low salt food has resulted in an improvement in FMD in normotensive overweight and obese adults with BMIs ranging from 27 to 40 (Dickinson et al., 2014b). It should be noted that FMD increased at day 2 in both the reduced salt and usual salt diet groups although only the reduced salt group reached statistical significance. Further, while FMD and BP improvements have also been seen after 2 weeks of a low sodium diet, this was not long enough to show any changes in arterial stiffness (PWV and AI) (Dickinson et al., 2009). Also, in two studies, the low sodium diet intervention was not compared to a subject's habitual intake but rather a prescribed high sodium diet (Dickinson et al., 2009; Jablonski et al., 2013b). Assessment of the habitual sodium

intake is important because there could be variation in the results from those who normally consume higher sodium levels versus lower sodium levels. In the Dickinson et al (2014b) study, baseline sodium intake varied significantly (2761 ± 1031 mg) and could have had an unintentional effect on the results. Those who consumed lower levels of sodium could possibly have had better vascular function than those who had higher intakes. Further, in the Jablonski et al., (2013b) study, all subjects consumed a low sodium diet and were supplemented with sodium or placebo tablets. Therefore, subjects were not making holistic changes to their habitual intake. A period of 4 weeks has shown improvements in blood pressure and endothelial function assessed via FMD (Jablonski et al., 2013b) in a group of older adults with hypertension. Longer interventions have shown high subject dropout rates (50%) without any additional improvements seen after the longer time period (Dickinson et al., 2014b). It has not been seen before if dietary sodium reduction achieved solely through behavior change and dietary counseling would produce similar effects seen in these counseling combination studies.

1.6 Innovation

The novelty of this study is its' real world application. Studies that provide us with information on the benefits of a low sodium diet on blood vessel function come from "controlled feeding studies". This means that the subjects in these studies do not make their own dietary choices, but rather are provided with a week or several weeks' worth of prescribed food and drink. These studies ensure the sodium intake levels are accurate and consistent. Less work has focused on the use of dietary counseling as a means to get high sodium consumers to eat less sodium and examining whether or not this results in an improvement in blood vessel health. This is the next step in this area.

Translating the findings of this study into clinical recommendations for the US population is desired.

1.7 Aim and Hypotheses

Cardiovascular disease is the leading cause of death in the United States (Kochanek et al., 2011). A diet high in salt has been linked to CV mortality (Umesawa et al., 2008), high blood pressure (Drenjancevic-Peric et al., 2011; INTERSALT, 1988; Todd et al., 2010), and endothelial dysfunction (Dickinson et al., 2011; DuPont et al., 2013). Endothelial dysfunction is a risk factor for CVD and a precursor to atherosclerosis (Anderson et al., 1995; Shimokawa, 1999). The average sodium intake in the U.S. is approximately 3,400 mg/day and well above the recommended intake of 2,300 mg or 1,500 mg for special populations (Centers for Disease Control and Prevention (CDC), 2011). Reductions in dietary sodium intake have demonstrated improvements in endothelial function in overweight/obese normotensive (Dickinson et al., 2009; Dickinson et al., 2014b) and pre-hypertensive individuals (Jablonski et al., 2013b). Some of these studies utilized dietary counseling by a registered dietitian or nutritionist to achieve participants desired sodium intakes. By utilizing dietary counseling as a technique to manipulate sodium intake, clinical findings can be translated to a larger scale of people. It is unknown whether a dietary sodium reduction achieved through dietary counseling to 2g/day in habitually high sodium (>5 g sodium/day) consumers who are free of hypertension and obesity will result in improved endothelial function.

Therefore, the following aim and hypotheses was proposed:

Specific Aim: To determine if dietary counseling to lower sodium intake to 2 grams/day in habitually high sodium consumers (≥ 5 g/day) results in an improvement in vascular function.

Hypothesis 1: Habitually high sodium consumers (≥ 5 g/day) who are counseled to achieve a 2g/day sodium diet for a period of 30 days will have improved endothelial function as assessed by brachial artery FMD at day 30 compared to baseline.

Hypothesis 2: Habitually high sodium consumers (≥ 5 g/day) who are counseled to achieve a 2g/day sodium diet for a period of 30 days will have lower arterial stiffening and wave reflection as assessed by PWV and AIx at day 30 compared to baseline.

Hypothesis 3: Habitually high sodium consumers (≥ 5 g/day) who are counseled to achieve a 2g/day sodium diet for a period of 30 days will have greater NO-mediated cutaneous vasodilation in response to local heating at day 30 compared to baseline.

Hypothesis 4: Habitually high sodium consumers (≥ 5 g/day) who are counseled to achieve a 2g/day sodium diet for a period of 30 days will have decreased cutaneous vasodilation in response to infusion of the antioxidant Ascorbic Acid at day 30 compared to baseline.

Chapter 2

RESEARCH DESIGN AND METHODS

The University of Delaware Human Subjects Review Board has approved all procedures (Appendix H).

2.1 Subjects

Eight habitually high sodium consumers (consumption \geq 5g/day) ages 22-65 were recruited to participate in this study. These subjects were recruited from a database of subjects at the University of Delaware who agreed to be contacted again for future studies as well as high sodium consumers from the surrounding University of Delaware community. Out of fifteen subjects who were consented, eight subjects met the inclusion criteria for being habitually high sodium consumers and participate in the dietary intervention.

Inclusion Criteria

We recruited healthy adults for participation. Subjects were currently consuming a habitually high sodium intake (sodium consumption \geq 5g/day) assessed from a 3-day diet record in order to be eligible. All subjects were able to speak English, read and write, have functional capabilities such as speaking, hearing, and good eye vision in order to actively participate in the counseling visits. Subjects were

withdrawn from the experiment if unable to complete a satisfactory 3-day food record prior to the study period or during any of the (5) diet recalls.

Exclusion Criteria

Subjects were excluded if they consumed a habitual sodium intake of <4.5 g per day. Additionally, subjects were excluded if they had diabetes, kidney disease, high blood pressure ($\geq 140/90$ mmHg), coronary heart disease, a history of stroke, arrhythmias, peripheral vascular disease, lung disease, obesity (as defined by a BMI ≥ 30), or were current smokers as assessed by a medical history questionnaire. Also, the use of any medications to treat those conditions excluded participation. Pregnant and breastfeeding women, and high-level endurance athletes were excluded as well.

2.2 Screening Visit

The screening visit took place at the Nurse Managed Health Center (NMHC) at the STAR Campus and lasted approximately 45 minutes to 1 hour. Prior to this visit, subjects' were consented, and determined to be habitually high sodium consumers by filling out a three day food record. Subjects arrived fasted from food, alcohol, and caffeine for 12 hours, with no physical activity for at least 24 hours prior to this visit. During the screening, the informed consent was reviewed with the subject along with the study protocol. After consent was given, a medical history questionnaire was completed (Appendix A). The screening visit included a blood draw, resting blood pressure measurement (GE Medical Systems, Dinamap Dash 2000, Milwaukee, WI), a resting 12-lead electrocardiogram (Schiller AT-10, Electra-Med, Flint, MI), a physical examination performed by a registered nurse, and collection of height and weight for determination of subjects' body mass index (BMI) (Healthometer Scale, Continental Scale, Bridgeview, IL). The blood sample was analyzed for complete blood count

(CBC), comprehensive metabolic profile, and lipids. A physical activity questionnaire was also completed at the screening visit to determine the subjects' level of physical fitness (Appendix I). This questionnaire was given to the subject at the baseline testing visit if the time between screening and baseline visit was >1 month to confirm physical activity had not changed. Prior screening visit, subjects were provided with instructions on how to keep a three-day food record (Appendix B), the Food Models for Estimated Portions (FMEP) guide, as well as a blank food log (Appendix C) that was completed prior to the dietary counseling visit to ensure that they were habitually high sodium consumers.

2.3 Experimental Setup

Following screening, subjects underwent baseline testing. The testing procedures were completed while the subject was consuming their habitual diet. This was done in order to assess vascular health on their habitual intake prior to the dietary intervention. Subjects arrived at the laboratory after a 12-hr overnight fast and underwent vascular function testing (to be described later). Upon completion of the baseline testing visit, subjects participated in a counseling and education session with a Registered Dietitian (RD), who counseled them to consume a 2-g sodium diet. Day 0 of the study period began when the subject started the 2-g sodium diet, and this sodium level was maintained throughout the entire 30-day study period. During the 30-day study period, subjects participated in 1 dietary recall interview each week, totaling 5 dietary interviews. Subjects came in for a midpoint visit during the 30 days, upon completion a 24-hour urine collection and 24-hour blood pressure cuff collection, to drop these items off and meet with the RD for an in person 24-hour recall. At the end of the 30-day study period, subjects underwent the same vascular

testing measurements as were performed at the baseline testing visit. See **Figure 1** below for more detail on the timeline.

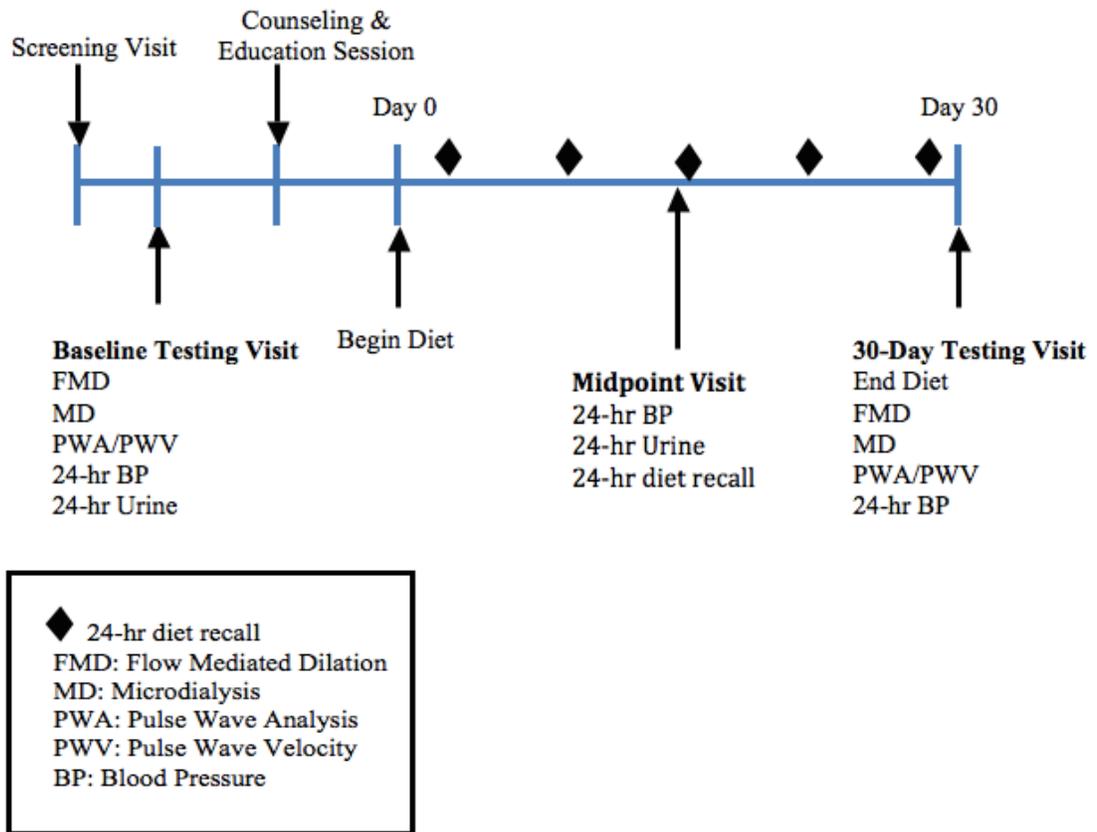


Figure 2.1: Timeline of Experimental Design

2.4 Dietary Education and Counseling

Dietary education and counseling was individualized for each subject and provided prior to the 30-day study intervention by the RD. The session was a one-on-one, in-person session lasting between 1 to 1 ½ hours. During this session, subjects

were instructed to achieve a 2g-sodium diet every day for 30 days. This was achieved by focusing on the principles of the DASH diet, educating subjects on label reading, and providing take home materials on reducing dietary sodium (See Appendix E). The RD used the 3-day food record provided from the subject to make specific recommendations on dietary choices. Participants were encouraged to schedule weekly in-person sessions at this visit with the RD to review dietary history, provide help and assess compliance. Depending on the clients' preferences and schedule, a weekly diet recall interview was performed either in person or over the telephone, totaling 5 dietary recall interviews during the study period (see Appendix F).

2.5 Dietary Assessments

Subjects kept a 3-day food record prior to beginning the study period to assess their habitual diet, and completed five dietary recall interviews after receiving dietary counseling and education in order to assess dietary intake. Previous studies have used similar methods of assessing diet intake prior to study period, and gathering objective and subjective dietary information to ensure compliance during the study period (McMahon et al., 2013; Todd et al., 2010). Subjects were then provided with written instructions and a 3-day food log to fill out while consuming their normal diet (see Appendices B&C). The food record is a prospective dietary assessment technique where subjects recorded all food and beverages consumed on two days a week and one weekend day, along with preparation method. This 3-day food record was used to analyze the nutritional components of the habitual diet, assess typical eating habits, and to formulate changes that the subject will be able to make for use in the Dietary Counseling and Education Session (see above). The USDA Food Models for

Estimating Portions 2007 (FMEP-2007) booklet was provided to each subject in order to successfully fill out the 3-day food record, as well as for use in conducting the dietary recall interviews. The USDA FMEP booklet is a set of 30 drawings used to help estimate food and drink portion sizes consumed. This is an accurate and reliable method used in conducting 24-hr recalls.

There were a total of five 24-hr recalls conducted during the study period. These 24-hr recalls were conducted to assess diet compliance, assess barriers to achieving the 2-g sodium diet, and to direct further counseling to improve adherence if needed. A 24-hr recall is a retrospective list of all foods and beverages consumed in the previous 24-hrs; this was defined as “midnight to midnight”. These 24-hr recalls were conducted either in person or over the telephone using the USDA Food Models for Estimating Portions Booklet to increase accuracy. The midpoint recall was performed in person. This method is easy to administer, and requires little effort on the subjects’ end. Although a 24-hr recall may not be representative of a persons’ usual intake, this can be overcome by performing multiple diet recalls over various nonconsecutive days to gain a better understanding of their intake.

Use of both a 3-day food log and multiple 24-hr recalls provided the most information for the researchers to examine. Information was focused on ensuring compliance with the 2g/day sodium diet.

The Nutrient Analysis System Nutrition Data System for Research (NDS-R) from the University of Minnesota was used to analyze subjects’ 3-day food records and each of their 24-hr recalls during the entire study period. NDS-R is a Windows-based analysis program with multiple functions; it can analyze 24-hr recalls, food records, menus, and recipes after manually inputting the information. The calculation

of nutrient composition provided data per ingredient, food, meal or day in a report format or analysis file format. Supplements may also be evaluated and quantified using the NDS-R software (Reagents of the University of Minnesota, January 13, 2014). Habitual sodium intake from the 3-day food record was above 5g/day sodium. The five separate 24-hr recalls were analyzed for sodium content in order to ensure the subject is maintaining their reduced level of 2g/day sodium intake.

2.6 Testing Days

The subjects arrived for the vascular testing visits fasted for 12 hours, including no alcohol or caffeine and no exercise in the preceding 24 hours. The subject arrived wearing or brought a pair of shorts and a t-shirt in order to accurately perform assessment of blood vessel function. Any premenopausal women were assessed in the early follicular phase of their menstrual cycle.

Anthropometrics were measured at these visits while the subject was fasted, before the vascular measurements. Subjects' height was measured using a wall-mounted stadiometer to the nearest 0.1 cm, barefoot and feet flat on the floor; weight and body composition was assessed using a Bioelectrical Impedance Analyzer (BIA) (Bodystat 1500MMDD) scale to the nearest 0.1kg with the subject wearing light clothing and barefoot. Using height and weight, body mass index (BMI; kg/m^2) was calculated for each subject. The scale also provided information on body fat percentage and body composition.

2.6.1 Cutaneous Microvascular Function

During the entire visit, subjects were resting in a semi-recumbent position. Instrumentation to determine cutaneous microvascular function was performed first. Using sterile techniques, the subjects were instrumented with two intradermal microdialysis fibers (MD 2000, Bioanalytical Systems) with a membrane length of 10 mm and a 20-kDa cutoff of the ventral side of the nondominant forearm for localized delivery of pharmacological agents, as described previously (DuPont et al., 2011; Dupont, Farquhar, Townsend, et al., 2011). A trained researcher who has experience using this technique placed the MD fibers. A tourniquet was placed around the upper arm to increase visibility of superficial veins. Two pairs of pen marks were made on the forearm approximately 2.5 cm (1 inch) apart, indicating where the fibers were to enter and exit. The site was then cleaned using Betadine and alcohol, followed by 10 minutes of ice to anaesthetize the area. Upon an acceptable level of anesthesia, a 25-gauge needle was inserted into the skin at entry and exit points marked on the skin. The microdialysis fibers were threaded through the lumen of the needle, and subsequently removed upon placement of the semi-permeable membrane of the fiber. At this point, a laser Doppler probe and its holder were taped over each site to determine red blood cell flux. The probes were then placed in a local heater (MoorLAB, Temperature Monitor SH02; Moor Instruments, Axminster, UK), which was then affixed to the skin directly above each microdialysis membrane. The temperature was varied throughout the experiment, and started at 33°C (91.4°F). Lactated Ringers solution was run through the fibers first, waiting for hyperemia to subside, which took anywhere between 60 and 90 minutes.

During the local heating protocol, 20 minutes of baseline data was collected. The following pharmacological agents were delivered to the two sites: 1) 2 μ M/min Lactated Ringer's solution; and 2) 20 mM Ascorbic Acid (Vitamin C). The MD fibers were then infused at 2 μ l/min for at least 30 minutes at a baseline temperature of 33°C. This standard, non-painful heating protocol was used to induce NO dependent vasodilation (Kellogg, Liu, Kosiba, & O'Donnell, 1999; Minson, Holowatz, Wong, Kenney, & Wilkins, 2002). After baseline measurements, temperatures were increased 0.5°C every 5 seconds to a temperature of 42°C and remained here throughout this local heating protocol. After the red blood cell flux reached a stable plateau (~ 40 minutes), 10mM *N*^G- nitro-L-arginine methyl ester (L-NAME; Sigma-Aldrich, St. Louis, MO, USA) was infused at 2 μ l/min through the control (Lactated Ringers) and treatment site to quantify NO-dependent vasodilation. The red blood cell flux then reached a new stable plateau, at which point the local heaters were set at 43°C (108°F), and 28 mM of sodium nitroprusside (SNP) (Nitropress; Hospira Inc.) were infused at the sites at a rate of 2 μ l/min. All pharmacological solutions were mixed immediately prior to usage, dissolved in lactated Ringer solution, and filtered using syringe microfilters (Whatman Puradisc 13 mm Syringe Filgers, Florham Park, NJ, USA). The solutions were also wrapped in foil to prevent photodegradation of the agents.

The microdialysis procedure concluded with the cleaning and removal of fibers from the skin. The skin was cleaned with alcohol at entry and exit points for the fibers, and then the fibers were carefully extracted from the skin by an experienced researcher competent in this technique. The forearm was then covered with a sterile bandage and iced for 10 minutes if necessary.

Cutaneous red blood cell (RBC) flux- an index of skin blood flow- was measured at the beginning of the MD procedure using an integrated laser-Doppler flowmeter probe placed in a local heater (MoorLAB, Temperature Monitor SH02, Moor Instruments, Devon, UK) on the skin directly over each microdialysis fiber membrane. The outcome measure from this specific part of the procedure was the cutaneous vascular conductance (CVC), which was calculated as RBC flux divided by mean arterial pressure. Baseline and the plateau CVC were obtained over a stable 10-minute period. Initial peak and nadir CVC was calculated by averaging the highest and lowest values, respectively, over a stable 60 s period. The data collected here was expressed as a percentage of maximal CVC obtained during sodium nitroprusside infusion (%CVC_{max}). Blood pressure was also obtained simultaneously over 10 minutes using an automated oscillometric sphygmomanometer (Dinamap Dash 2000, GE Medical Systems) while measuring the RBC flux. Heart rate and blood pressure was collected on the arm without the microdialysis fibers through a single lead ECG and the blood pressure cuff.

2.6.2 Brachial Artery Flow-Mediated Dilation

Brachial artery flow-mediated dilation was used to assess endothelium-dependent dilation according to established guidelines (Corretti et al., 2002). Endothelium dependent dilation is the vasodilatory response to hyperemia in the conduit brachial artery and was assessed at the baseline and day 30 testing visits. Three self-adhesive electrodes were placed on the subjects' chest, followed by a 15-minute period of rest to relax and normalize heart rate. The subjects were seated supine, with the right arm lying straight, flat out to the side on top of a pillow at heart level. The palm of the hand and wrist were facing up. A narrow blood pressure cuff

was placed on the proximal forearm, 3-cm below the antecubital crease. Longitudinal images of the brachial artery and continuous Doppler blood velocity were obtained using a 10 MHz linear phased array ultrasound transducer (Logic e, GE).

After the 15-minute rest, baseline artery images were collected for 1 minute, at which point the blood pressure cuff (AG101 rapid cuff inflator, Hokanson, Bellevue, Washington, USA) was inflated to 200 mmHg for 5 minutes, causing blood flow to the forearm to cease. Then the cuff deflated and an image was recorded for 2 minutes. Images and blood velocity were recorded during the last 15s of occlusion and continued for 2 minutes following cuff release for the determination of peak diameter change and calculation of the shear rate. Cuff position and artery are very important to keep consistent (Hodson et al., 2010; Thijssen et al., 2011); the researcher marked on the arm point at which the cuff was placed and the brachial artery was found. FMD was used as a measure of endothelial dependent function and expressed as a percent change from baseline normalized shear rate calculated from the blood flow velocity and vessel diameter data. Measurement of brachial artery FMD can be influenced by dietary intake, recent aerobic or resistance exercise, caffeine and alcohol ingestion, and supplement/medication use (Thijssen et al., 2011). Time of day brachial artery FMD was also be standardized as much as possible to reduce variability.

2.6.3 Pulse Wave Analysis

Pulse wave analysis was used to determine wave reflection as assessed by augmentation index (AIx). A central aortic pressure waveform was synthesized from the measured brachial artery pressure waveform with the SphygmoCor Px system (AtCor Medical, Sydney, Australia), which uses a generalized transfer function. Central pressures, AIx, and the time delay of the reflected wave (*TR*) were obtained

from the synthesized wave. Augmentation index is defined as the ratio of reflected wave amplitude and pulse pressure and is calculated as $(P_s - P_i)/(P_s - P_d)$ where P_s is peak systolic pressure, P_d is end diastolic pressure, and P_i is an inflection point marking the beginning upstroke of the reflected pressure wave. TR is the travel time of the forward wave from the heart to the major reflecting site and back. Pulse pressure amplification ratio was calculated as brachial pulse pressure/central pulse pressure. Mean arterial pressure (MAP) was calculated as brachial pulse pressure/central pulse pressure. MAP was calculated over the cardiac cycle from the calibrated radial pressure waveform.

2.6.4 Pulse Wave Velocity

Carotid to femoral PWV was measured by simultaneously recording carotid and femoral pressure wave after the subject had a 15-minute quiet rest in the supine position. Carotid-femoral PWV was measured by placing a cuff around the upper thigh to capture the femoral wave, and a tonometer was used to capture the carotid waveform (SphygmoCor Px, AtCor Medical, Sydney, Australia). The distance from the carotid measurement point to the sternal notch was subtracted from the distance from the sternal notch to the femoral measurement point and used as a propagation distance. PWV was calculated as propagation distance/time delay; it is a measure of regional stiffness of the aorta.

2.6.5 Endothelium Independent Dilation

To ensure that any observed changes in endothelium dependent dilation were due to the altered endothelial function and not the altered smooth muscle responsiveness, endothelium independent dilation was assessed. Endothelium independent dilation was determined on the right arm by measuring brachial artery

dilation in response to sublingual nitroglycerin (NTG, 0.4mg). Endothelium independent dilation was measured at the end of the vascular testing visits. Before the nitroglycerin tablet was given, three standing blood pressures were taken. If the systolic blood pressure was below 110 mmHg, then the nitroglycerin trial did not proceed. Further, endothelium independent dilation was not performed if the NMHC was not open. This was to ensure that sufficient medical staff was available as a precautionary measure. Subjects taking Viagra, Cialis, or Levitra were not given a nitroglycerin tablet, since subjects taking these drugs may have a greater decline in blood pressure during sublingual nitroglycerin administration. A baseline image of the brachial artery was recorded for one minute, and one tablet of 0.4 mg nitroglycerin was placed underneath the tongue of the subject. Images of the brachial artery were recorded for 10 minutes, while blood pressure readings were collected simultaneously every 2 minutes on the opposite arm. After the imaging was complete, the subject remained in the supine position for 30 minutes while blood pressure was monitored. Once the 30-minute rest was over, three more blood pressure measurements were taken until the subjects' blood pressure was within 10 mmHg of baseline measurements.

2.7 Urine and Blood Analysis

A 24-hr urine was collected at baseline, midpoint, and 30 days in order to provide electrolyte content as well as information on renal function. Subjects were provided with written and verbal instructions on how to collect their urine (Appendix D). The urine sample was analyzed for sodium, potassium, and chloride as well as plasma osmolality (EasyElectrolyte Analyzer, Medica, Bedford, MA; Model 3D3 Osmometer, Advanced Instruments, Norwood, MA). Some of the analyses were done

immediately in the lab, and some of the urine samples were stored in a freezer at -80°C for future analysis. All urine analyses were done in triplicate, right after calibration. Electrolytes were analyzed using the EasyElectrolyte Analyzer (Medica, Bedford, MA) after proper cleaning and standardizing the analyzer. A pipette was used to fill a micro-centrifuge tube with 100 µL of urine and 900 µL diluent, which was inserted into the analyzer for sodium, potassium and chloride content. Specific gravity was also analyzed using the Goldberg TS Meter Clinical Refractometer (Reichert Technologies, Depew, NY). Female participants were checked for pregnancy during each of the three 24-hour urine collection periods. In the event of a positive test, the subject was notified and referred to a physician.

A venous blood sample was taken at the vascular testing visits using sterile techniques and procedures by a trained phlebotomist. About 5 tablespoons (2.5 fluid oz) were sampled from the blood vessel on the arm in the antecubital space. The whole blood samples were used to measure hemoglobin (Hb 201p model; HemoCue, Lake Forest, California, USA), hematocrit (Clay Adams Brand, Readacrit Centrifuge; Becton Dickinson, Sparks, Maryland, USA), plasma osmolality (Advanced 3D3 Osmometer; Advanced Instruments), and serum electrolytes-sodium, potassium, and chloride (EasyElectrolyte Analyzer; Medica, Bedford, Massachusetts, USA).

2.8 Ambulatory Blood Pressure Monitoring

Participants' wore an ambulatory blood pressure cuff (90207 ABP Monitor, Spacelabs Healthcare, Issaquah, WA) after the baseline vascular testing visit, the midpoint visit, and the 30-day vascular testing visit. Written and verbal instructions were provided on how to wear the monitor (Appendix D). After entering in the appropriate participant characteristics, including when the subject woke up and went

to sleep, the cuff was placed on the dominant arm. The blood pressure cuff automatically took readings every 20 minutes during the day, and every 30 minutes at night.

2.9 Statistical Analysis

A student's paired t-test was used to assess if there was a difference in the % change of FMD, carotid to femoral PWV, and AIx between the baseline and the 30-day vascular testing visits. A student's paired t-test was performed to assess differences in the NO contribution to the plateau between habitual sodium intake and a 2g/day low sodium diet (baseline vs. 30 day), as well as ascorbic acid compared to the Ringer's site. Descriptive statistics are presented as means \pm standard error of the measure. Significance is set at $p < 0.05$.

Chapter 3

RESULTS

3.1 Subject Characteristics

Eight subjects (6M/2F) completed this research study examining the effects of a low-sodium dietary intervention (defined as 2,000 mg/d) on vascular function for 30 days. Out of fifteen individuals consented, only 8 individuals completed the dietary intervention, as not all met the inclusion criteria of consuming habitually high levels of sodium ($>5\text{g/day}$) or were able to commit to attending multiple testing visits at STAR campus. The age for this group of subjects ranged from 22 to 58 years, with an average age of 32.3 ± 5.6 years. On average, their BMI was $24.3 \pm 0.8 \text{ kg/m}^2$, indicating that on all subjects were within a normal weight range, and not obese.

Despite consuming high levels of dietary sodium, average blood pressure at screening was within the normal range (SBP: $131 \pm 3 \text{ mmHg}$; DBP: $78 \pm 2 \text{ mmHg}$) and subjects were free of hypertension (defined as $\text{BP} \geq 140/90 \text{ mmHg}$). Subjects had normal renal function and cholesterol levels that were in the normal range. Other subject characteristics are presented in Table 3.1.

Table 3.1 Subject Characteristics

<i>Demographic Characteristics</i>	
Age, yr	35.3 ± 5.6
Height, cm	174.14 ± 4.87
Weight, kg	72.5 ± 4.6
BMI kg/m ²	24.3 ± 0.8
<i>Hemodynamic Measurements</i>	
HR, beats/min	66 ± 4
SBP, mmHg	131 ± 4
DBP, mmHg	78 ± 2
MAP, mmHg	98 ± 4
<i>Renal Function</i>	
Blood urea nitrogen, mg/dL	13.4 ± 1.2
eGFR, ml·min ⁻¹ ·1.73m ²	100.7 ± 6.0
<i>Blood Chemistry</i>	
Total Cholesterol, mg/dL	200 ± 16
High-density lipoprotein, mg/dL	70.1 ± 6.3
Low-density lipoprotein, mg/dL	108.6 ± 12.5
Hemoglobin, mg/dL	15.0 ± 0.4
Hematocrit, %	45 ± 1
Glucose, mg/dL	91.1 ± 3.3

Values are means ± SE. BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure.

3.2 Habitual Dietary Intake Characteristics

The habitual dietary intake of subjects was assessed and select nutrients are presented in Table 3.2. The average energy intake was 3131 ± 290 kcal/d with 47% of

the energy coming from carbohydrates, 33% from fat, and 14% from protein. On average, habitual sodium intake assessed from the three-day food log was 5307 ± 337 mg/d (range 4475 to 7377 mg/d), indicating subjects were habitually high sodium consumers. Habitual potassium intake fell below the recommended dietary allowance (RDA) of 4,700 mg/d (ranged from 2193 to 6798 mg/d). Finally, calcium and magnesium intake was adequate and slightly above the RDA.

Table 3.2 Habitual Nutrient Intake

Nutrient	
Energy, kcal	3131 ± 290
Total Carbohydrate, g	371 ± 34
Total Fat, g	120 ± 12
Total Protein, g	108 ± 9
Na ⁺ , mg	5307 ± 337
K ⁺ , mg	3678 ± 547
Mg ⁺ , mg	478 ± 94
Ca ⁺ , mg	1155 ± 71
Caffeine, mg	187 ± 61
Free water, g	3324 ± 470
Alcohol, % energy	6 ± 2

Values are mean \pm SE. Ca⁺, calcium; K⁺, potassium; Mg⁺, magnesium; Na⁺, sodium.

3.3 Effects of a Low Sodium Diet on Daily Nutrient Intakes

Throughout the 30-day low sodium diet period, subjects underwent five 24-hour dietary recalls. These dietary recalls were performed either in person, or over the telephone to ensure that the 2g/day diet was being met. Over the 30-day period, the average sodium intake was at or below the 2 g/day goal for each of the five dietary recalls (see Table 3.3). Sodium and potassium intake reached their lowest average intake values at the midpoint dietary recall, which occurred halfway through the 30-day period; average sodium intake was 1111 ± 253 mg and average potassium intake was 2572 ± 223 mg. Sodium intake significantly differed at each dietary recall compared to habitual intake ($p < 0.05$), while there were no significant differences throughout the 30-day period. Potassium intake remained unchanged, while energy at diet recall 5 was significantly reduced compared to subjects' habitual energy intake.

Table 3.3 Nutrient Analysis from 5 Dietary Recalls

Nutrient	Recall 1	Recall 2	Recall 3	Recall 4	Recall 5
Energy, kcal	2611 \pm 180	2364 \pm 335	2142 \pm 181	2475 \pm 327	2039 \pm 245
Carbs, g	358 \pm 25	300 \pm 39	274 \pm 30	315 \pm 41	247 \pm 34
Fat, g	80 \pm 8	83 \pm 16	66 \pm 9	83 \pm 15	71 \pm 12
Protein, g	105 \pm 10	102 \pm 15	88 \pm 12	113 \pm 14	83 \pm 12
Na ⁺ , mg	1643 \pm 384	1772 \pm 353	1111 \pm 253	1889 \pm 213	1788 \pm 409
K ⁺ , mg	3978 \pm 548	3376 \pm 468	2572 \pm 223	3225 \pm 263	3047 \pm 397
Mg ⁺ , mg	538 \pm 108	432 \pm 55	327 \pm 40	440 \pm 46	374 \pm 21
Ca ⁺ , mg	1062 \pm 144	1194 \pm 205	908 \pm 188	1001 \pm 128	936 \pm 110
Free Water	3141 \pm 405	3075 \pm 336	2585 \pm 230	2847 \pm 204	3039 \pm 310
Alcohol, % energy	1.12 \pm 0.73	2.31 \pm 1.3	4.44 \pm 2.5	2.63 \pm 0.9	1.68 \pm 1.1

Values are mean \pm SE. Ca⁺, calcium; K⁺, potassium; Mg⁺, magnesium; Na⁺, sodium.

3.4 Effects of a Low Sodium Diet on Hemodynamic Measurements

3.4.1 Blood Pressure

Twenty-four hour ambulatory blood pressure readings are shown in Table 3.4 for baseline, midpoint, and day 30. There were no differences in blood pressure across the three time points. Mean arterial pressure at baseline and day 30 of the low sodium diet are shown in Figure 3.1 indicating there were no significant changes in MAP across the 30-day intervention.

Table 3.4 Ambulatory Blood Pressure Monitor Readings

	Baseline	Midpoint	Day 30
24-hr SBP, mmHg	121 ± 3	120 ± 2	121 ± 3
24-hr DBP, mmHg	73 ± 2	74 ± 2	74 ± 3
24-hr MAP, mmHg	88 ± 2	89 ± 2	89 ± 3
24-hr PP, mmHg	47 ± 2	45 ± 3	47 ± 1
24-hr HR, bpm	68 ± 2	67 ± 1	68 ± 2

Values are mean ± SE. DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure.

Baseline & Day 30 n=8; Midpoint n=6

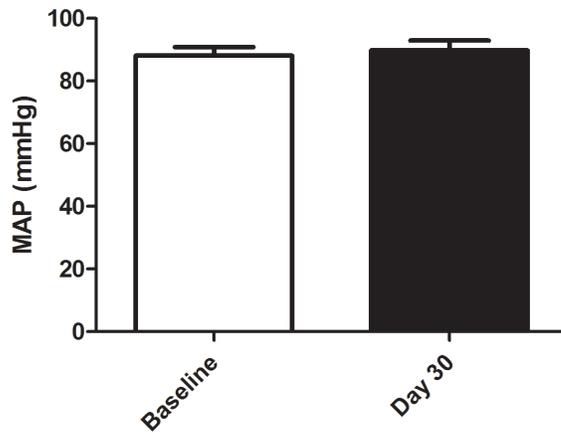


Figure 3.1 Mean Arterial Pressure (MAP) at Baseline and at 30 Days. Values are mean \pm SE.

3.5 Effects of a Low Sodium Diet on Biochemical and Anthropometric Parameters

3.5.1 Biochemical Measurements

The urinary excretion data is shown in Table 3.5. Twenty-four hour urinary sodium excretion significantly decreased from baseline to midpoint ($p < 0.05$), and trended toward significance between baseline and day 30 ($p = 0.073$). There was a non-significant increase in the 24-hr urinary potassium excretion from baseline to day 30 ($p = 0.068$).

Table 3.5 Urinary Values at Baseline, Midpoint, and Day 30

Measure	Baseline	Midpoint	Day 30
Urine Osmolality, mOsmol/kg H ₂ O	608 ± 101	438 ± 92	365 ± 68
Urinary Na ⁺ Excretion, mmol/24h	156.1 ± 20.3	85.5 ± 15.9*	99.4 ± 12.1
Urinary K ⁺ Excretion, mmol/24h	64.3 ± 9.6	63.5 ± 10.2	86.9 ± 14.6
Urine Volume, mL	1650 ± 294	1531 ± 320	2175 ± 341

Values are mean ± SE. K⁺, potassium. Na⁺, sodium; * p < 0.05 vs. baseline. Baseline & Day 30 n=8; Midpoint n=6.

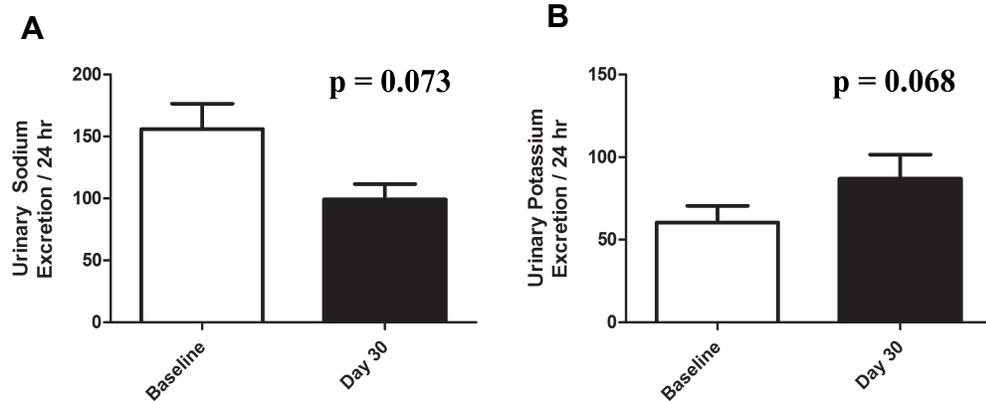


Figure 3.2 Urinary Excretion Values at Baseline and Day 30. Average Urinary Sodium Excretion (A); and Average Urinary Potassium Excretion (B) are shown. Values are mean ± SE.

The hematological values are shown in Table 3.6. There were no significant differences in any of the blood parameters between baseline and day 30 ($p > 0.05$).

Table 3.6 Anthropometric and Hematological Values

Variable	Baseline	Day 30
<i>Hematological</i>		
Hemoglobin, g/dl	14.0 ± 0.5	14.2 ± 0.2
Hematocrit, %	46 ± 1	45 ± 2
Serum Na ⁺ , mmol/L	138.3 ± 0.9	138.6 ± 0.6
Serum K ⁺ , mmol/L	4.5 ± 0.2	4.6 ± 0.2
Serum Cl ⁻ , mmol/L	102.3 ± 0.7	102.1 ± 0.9
Plasma osmolality, mOsmol/kg H ₂ O	290 ± 2	288 ± 2
<i>Anthropometrics</i>		
Weight, kg	75.5 ± 4.8	72.1 ± 2.9
Body Fat, %	23.0 ± 3.9	21.0 ± 3.8

Values are means ± SE. Cl⁻, chloride; K⁺, potassium, Na⁺, sodium.

3.5.2 Anthropometric Measurements

Subjects' anthropometrics at baseline and day 30 can be seen in Table 3.6. Over the thirty-day period, subjects average weight and body fat % remained unchanged.

3.6 Effects of a Low Sodium Diet on Vascular Function

3.6.1 Endothelial Function

To assess changes in endothelial function, brachial artery FMD was performed at baseline while subjects were consuming their habitual diet, and after consuming a 2g/day sodium diet for thirty days. At baseline, the average % change in brachial artery FMD was $7.29 \pm 1.06\%$ that rose to $7.80 \pm 1.5\%$ at day 30 for all subjects however this was not statistically significant ($p=0.66$; Figure 3.3a). When examining the individual results, we see that 5 of the subjects had an increase in brachial artery FMD. These 5 subjects were male while those that decreased following the low sodium diet were post-menopausal women and one male (Figure 3.3b). Figure 3.4 shows the brachial artery FMD responses of the male subjects alone. Their baseline FMD value of $6.98 \pm 1.43\%$ increased to $8.8 \pm 1.81\%$ at day 30 which trended towards significance ($p=0.13$).

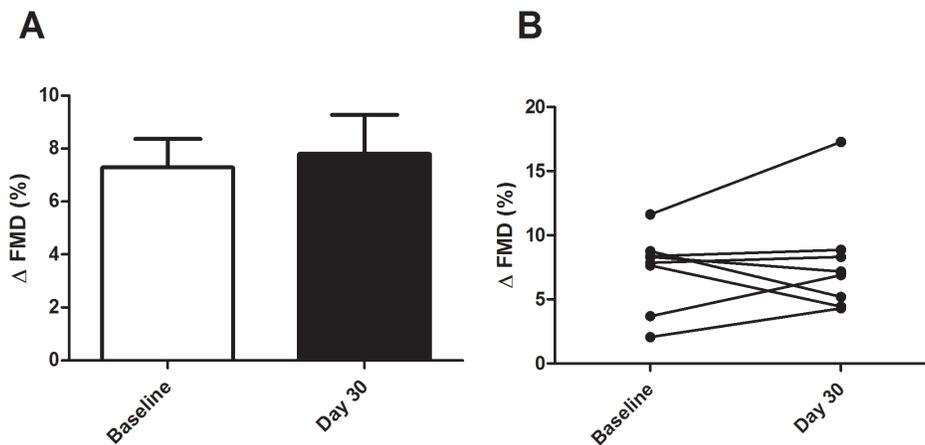


Figure 3.3 Brachial Artery Flow-Mediated Dilation at Baseline and at 30 Days in All Subjects. Average subject data (A) and individual data (B) are shown. Data shown in A are mean \pm SE.

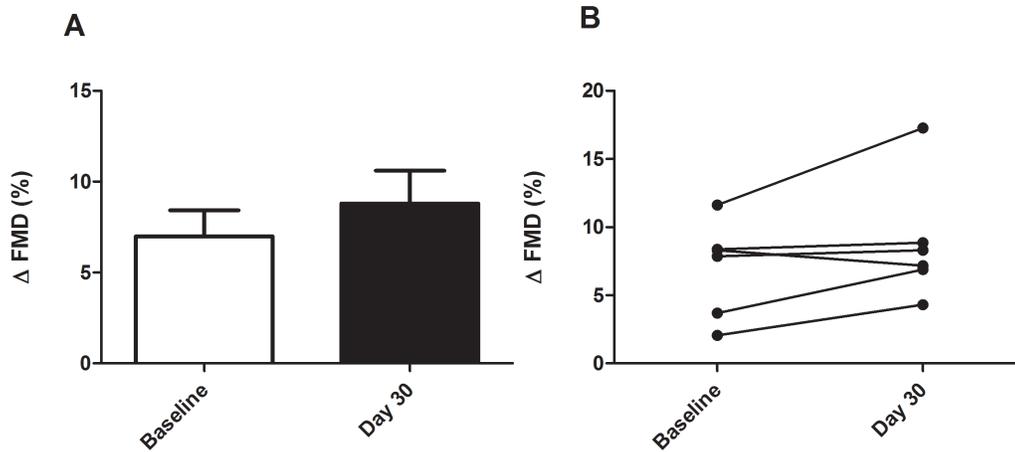


Figure 3.4 Brachial Artery Flow-Mediated Dilation at Baseline and at 30 Days in Male Subjects. Average subject data (A) and individual data (B) are shown. Data shown in A are mean \pm SE.

Brachial artery baseline diameter, peak diameter, and the change in brachial artery diameter did not significantly differ over time. Further, shear area under the curve (AUC) did not significantly differ over time during the low sodium diet.

Table 3.7 Brachial Artery Flow Mediated Dilation Parameters

Variable	Baseline	Day 30
Baseline Brachial Artery Diameter, cm	0.361 \pm 0.025	0.353 \pm 0.024
Peak Brachial Artery Diameter, cm	0.387 \pm 0.027	0.385 \pm 0.024
Brachial Artery FMD, Δ cm	0.026 \pm 0.005	0.032 \pm 0.005
Shear, AUC	14,203 \pm 3023	19,437 \pm 3567

Values are mean \pm SE; AUC, area under the curve

Endothelium independent dilation was only performed in one subject at both baseline (44.25%) and day 30 (38.28%). Hence, no statistical analysis could be performed.

3.6.2 Arterial Stiffness and Wave Reflection

To assess changes in stiffness, PWV and AIx were performed. PWV did not significantly differ over time (n=6; p=0.89). PWV was 6.28 ± 0.57 m/s at baseline and 6.38 ± 0.43 m/s at day 30 (Figure 3.5a). Further, AIx did not significantly differ over time in all subjects (n= 8; p=0.94; Figure 3.5b). AIx at baseline was $6.3 \pm 2.23\%$ and at day 30 was $6.95 \pm 2.46\%$. Further, when examining the male subjects alone, no differences were seen for PWV or AIx.

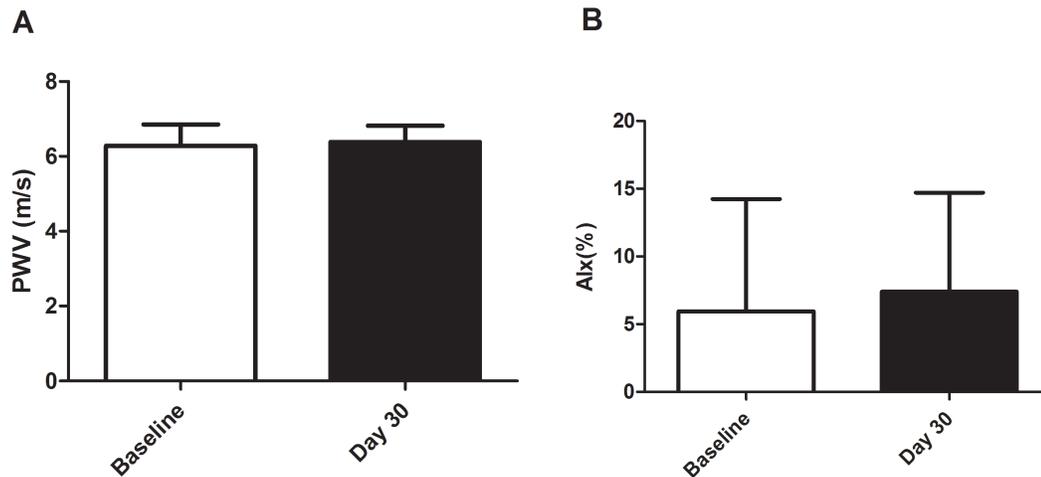


Figure 3.5 Pulse Wave Velocity (A) and Wave reflection (B) at Baseline and 30 Days. Data shown are mean ± SE.

3.6.3 Microvascular Function

Microvascular function data is shown in Table 3.8 and Figure 3.6. Absolute maximal CVC did not significantly differ between baseline and day 30 at either site suggesting maximum dilation did not differ in the skin microcirculation over time (Table 3.8). There was a significant difference in the baseline %CVCmax at the Ringers Site between baseline and day 30 ($p < 0.05$; $n = 7$); baseline %CVCmax significantly differed between the Ascorbic Acid and Ringers site ($p < 0.05$; $n = 8$). The NO-mediated plateau phase of the cutaneous hyperemia was unchanged in the Ringers site at day 30 suggesting no improvement in microvascular function with the sodium reduction. When separating out males from females, there were no significant differences found either. Finally, the plateau phase showed a trend towards significance at baseline in response to infusion of Ascorbic Acid ($p = 0.10$; Figure 3.6).

Table 3.8 Absolute Maximal Cutaneous Vascular Conductance

Drug	Baseline	Day 30
Ringers ^a	2.21 ± 0.28	1.87 ± 0.30
Ascorbic Acid ^b	1.72 ± 0.34	1.89 ± 0.33

Values are means ± SE. Expressed as red blood cell flux/mmHg. a: $n = 7$; b: $n = 6$

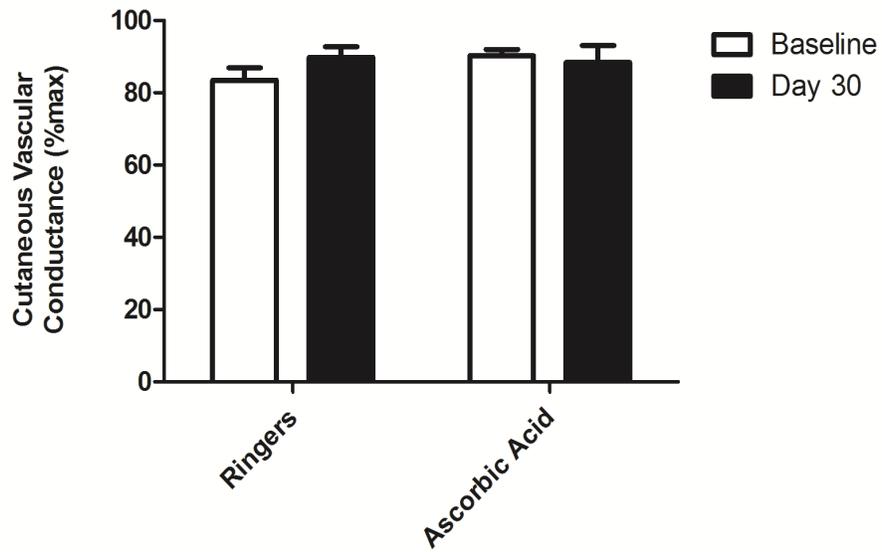


Figure 3.6 Plateau Responses at Baseline and Day 30. Values are mean \pm SE.
Ringers: n= 7; Ascorbic Acid: n= 6.

Chapter 4

DISCUSSION

The purpose of this study was to determine the effects of dietary counseling to achieve a 2-g/day low sodium diet for 30 days in habitually high sodium consumers (≥ 5 g/day) on vascular function. We hypothesized that endothelial function would be improved, as well as arterial stiffness and distensability. The primary finding of this study indicates that dietary counseling was an effective tool in lowering sodium intake in habitually high sodium consumers for 30 days. While sodium intake was reduced, we did not see a significant improvement in vascular function in this small sample of subjects. Brachial artery FMD, an assessment of conduit artery function, did not significantly change from baseline to day 30. However, when the male subjects were assessed alone, an improvement in brachial artery FMD that trended towards significance was seen. Further, thirty days of a low sodium diet did not change arterial stiffness as assessed via PWV or wave reflection as assessed by AIx. Finally, we hypothesized that microvascular function would be improved as seen by greater NO-mediated cutaneous vasodilation in response to local heating and further, cutaneous vasodilation would be improved at baseline in response to infusion of ascorbic acid compared to day 30. We found no significant differences between baseline and day 30 in the NO-mediated plateau at the Ringer's site however there was a trend towards significance at baseline with infusion of the antioxidant Ascorbic Acid suggesting that NO-mediated vasodilation was improved with ascorbic acid during a high sodium diet.

4.1 Sodium Intake

The subjects in this study successfully lowered their sodium intake from habitually high levels (5307 ± 337 mg) to $< 2,000$ mg (1788 ± 409 mg) by day 30 of the study period. This is noteworthy, as it is estimated that adults in the US consume about 3,400 mg/day of sodium (USDA & USDHHS, 2010), demonstrating that our subjects' sodium consumption was 1.5 times higher than the national average. We chose to individually counsel our subjects to achieve a 2,000 mg/day level of sodium, as it is very close to the recommendations set by the 2010 U.S. Dietary Guidelines for Americans (DGA), and is consistent with the sodium restriction used in other studies (Dickinson et al., 2009; Dickinson et al., 2014b, Jablonski et al., 2013b). The DGA recommends an intake of less than 2,300 mg, with a further reduction of to 1,500 mg for certain populations. Given that our subjects are not considered a special population, we chose 2,000 mg to ensure we met the 2,300 mg guideline. We were successful in our counseling intervention as subjects were able to keep their sodium intake levels on average below 2g/day consistently for the 30-day period, as indicated by the five dietary recalls.

4.1.1 Nutritional Assessment Methods

During our study, we used a three-day food record to assess subjects' habitual dietary intake prior to the intervention, and conducted five 24 hour diet recalls over the 30-day intervention. Other dietary counseling studies have used dietary intake records and 24 hour recalls to assess nutrient intake (Dickinson et al., 2009; Dickinson et al., 2014b). Dickinson et al., (2009) used three-day food records at the end of each 2-week intervention period to assess sodium and nutrient intake in their subjects while we

chose to use multiple 24-hr recalls to assess our subject's intake during our intervention.

Our decision to use multiple 24-hour recalls during the intervention was made for several reasons. Multiple 24-hr recalls can increase the accuracy of subject reporting. Studies have shown the accuracy of 24-hr recalls in men (Conway et al., 2004) as well as in obese and non-obese women (Conway et al., 2003). Second, we wanted to ensure compliance with the low sodium diet as well as assess any barriers to change, and thirdly, 24-hr recalls are less burdensome for subjects, and can be done over the phone or in person.

In addition to the 24-hour recalls, we did use a three day food record to assess habitual intake. Having a combination of the two dietary assessment methods has been shown to increase accuracy, as there are slight nutrient discrepancies between the two methods when compared (Bingham et al., 1994). Specifically looking at sodium intake, a study by Rhodes et al., (2013) demonstrated the accuracy of dietary recalls in reporting sodium intakes that are comparable to 24-hr urine excretion values.

Finally, our intervention ran over 30 days. Previous studies have used timeframes of 2 weeks (Dickinson et al., 2009), 4 weeks (Jablonski et al., 2013b), and 6 weeks (Dickinson et al., 2014b) and have shown improvements in endothelial function after a sodium reduction in prehypertensive (Jablonski et al., 2013b) and hypertensive individuals (Dickinson et al., 2009; Dickinson et al., 2014b). We chose thirty days as it has demonstrated on average a 95% subject retention rate during studies with similar time frames (Hodson et al., 2010; Sacks et al., 2001; Svetkey et al., 2004). During this study, we had no subjects drop out, leading to a 100% retention rate. Further, we wanted to test our females over the course of one menstrual cycle as

FMD has been shown to alter throughout the cycle (Williams et al, 2001). Studies examining endothelial function in response to low sodium diets have been done over a longer timeframe such as 12 weeks and have shown improvements (Dickinson et al., 2014b). However, given the number of shorter term studies and our desire to test all females during the same phase of the menstrual cycle, we chose 30 days.

4.1.2 Dietary Counseling to Lower Sodium Intake

Several studies have used dietary counseling as a tool, however, only one study closely mirrored our diet design as others have used sodium tablets to achieve high sodium levels (Dickinson et al., 2014b), or made comparisons of a low salt diet to a prescribed usual salt diet (Dickinson et al., 2009), rather than to habitual intake. Our study is similar to other counseling studies in which subjects meet with a dietitian, or nutritionist at the beginning of the intervention in order to learn how to follow the prescribed sodium diet (Dickinson et al., 2009; Dickinson et al., 2014b; Jablonski et al., 2013b). Jablonski et al., (2013b) provided subjects with an initial dietary counseling and education session, and had each subject complete a three-day diet record at the end of each 5-week intervention periods. Sodium tablets were used in this study to achieve a “normal sodium” diet, while placebo pills were given during the “low sodium” diet to blind the subjects’ to which diet they were on. During the Dickinson et al., (2009) study, subjects were provided with low salt bread and salt free butter to aid in compliance, but other than that they made dietary changes entirely on their own. Another study by Dickinson et al., (2014b) was longer term, and had subjects follow a low sodium diet for an entire 12 week period while consuming placebo or sodium tablets to achieve desired sodium levels. Our study is unique in that

we had a relatively short-term intervention (30 days) in which subjects made dietary changes entirely on their own, and maintained this for thirty days. Unlike other studies that compared the low sodium diet to a controlled high sodium diet, we compared our low sodium diet to subjects' own habitual diet. We found that all of the subjects were able to successfully make changes in their diet, and significantly lower their sodium intakes compared to their habitual diet.

4.2 Endothelial Function

Endothelial function was assessed at baseline and day 30 of our dietary counseling intervention. The baseline assessment was made while subjects were consuming their habitual diet. Baseline brachial artery FMD values were $7.29 \pm 1.06\%$ that is consistent with other studies of healthy, normotensive adults (Jablonski et al., 2009; Pierce et al., 2009) and in our lab in adults under high sodium conditions (DuPont, et al., 2013).

Improvements in brachial artery FMD from baseline to day 30 were not significant in this study when men and women were grouped together ($p=0.66$), however when men were evaluated separately, the improvements in FMD were trended towards significance ($p=0.13$) suggesting sodium reductions may have a beneficial effect on endothelial function in men. Dietary intervention studies have shown improvements in endothelial function in periods ranging from 2 to 6 weeks (Dickinson et al., 2009; Dickinson et al., 2014b; Jablonski et al., 2013b). These studies used dietary counseling in addition to provision of minimal foods to aid in achieving the prescribed diet.

While several studies have found an improvement in FMD as opposed to our study, there are several potential reasons for this discrepancy including study population, number of subjects, and differences in the sodium content of the diets. Dickinson et al., (2009) demonstrated a ~30% increase in FMD after consuming 2 weeks of 50 mmol/d vs. 150 mmol/d sodium. In contrast, our study took individuals from their habitual diet (230 ± 15 mmol/d) and lowered their sodium intake to on average ≤ 2 g/d (71 ± 6 mmol/d). Also, this study included relatively older (52.7 ± 6.0 yrs) and on average obese (BMI 31.6 ± 2.8) individuals, while our subjects were younger (35.3 ± 5.6 yrs) and non-obese (BMI 24.3 ± 0.8). There is evidence that endothelial function worsens as individuals' get older (Shimokawa, 1999), which is possibly why we didn't see as great as changes in FMD as a study in which older individuals are examined.

A study by Jablonski et al. (2013b) demonstrated a 68% increase in FMD after 4 weeks of a dietary sodium restriction. Subjects in this study were older, and pre-hypertensive while our subjects were younger and free of hypertension. This was a 10 week intervention study where subjects served as their own control. Subjects FMD was compared at the end of a 4 week usual sodium (150 mmol/d) and reduced sodium (50 mmol/d) diet. Hence, the subjects' usual habitual intake was not addressed in this study where we sought to improve our subject's current dietary intake to a lower sodium intake. Finally, this study had 17 subjects, compared to the 8 in our study.

A study by Dickinson et al., (2014b) utilized dietary counseling in overweight and obese subjects to demonstrate improvements in FMD in just 2 days during a 6 week period. All subjects were instructed to lower their sodium intake to ~100 mmol/d for 12 weeks. Subjects consumed a reduced salt diet for 6 weeks, followed by a diet

supplemented with 60 mmol/d of sodium tablets (usual salt) for 6 weeks. Subjects demonstrated an improvement in FMD compared to baseline after 6 weeks on the reduced salt diet, but not in PWV or AIx. This study differed from ours as measurements of FMD, PWV and AIx at the end of the two different diets (e.g. reduced salt and usual salt) were compared to baseline. Also, subjects in this study reduced their sodium intake to a greater extent than what was expected (goal: 100 mmol/d vs actual: 75 mmol/d).

While we did not see significant differences in FMD in our study, the men appeared to trend towards significance. In a study by Lennon-Edwards et al., (2014) dietary salt loading was shown to have a more detrimental effect on men than on women. Interestingly enough, this study demonstrated that when men and women were salt loaded for 1 week, brachial artery FMD was significantly lower in males than females. These results are similar to those seen in this study, where baseline male FMD was lower (6.98%) compared to female FMD (8.197%) while consuming habitually high levels of sodium. We saw that with a sodium reduction, male endothelial function improved (8.8%) while female FMD declined (4.82%). These differences could possibly be due to males' being more sensitive to changes in dietary sodium intake and hence, they responded more significantly to the intervention. While the women did not respond to the 2 g/d sodium diet, it should be mentioned that both women were post-menopausal and there were only two. It has been shown that pre-menopausal women exhibit greater cardio protection than men; this may be due to estrogen's ability to up regulate NO synthesis, as well as its' involvement in forming new blood vessels, and decreasing ET-1 and angiotensin II production (Perez-Lopez et al., 2010).

A study by Eisenach et al., (2012) demonstrated that males and females respond differently to dietary sodium perturbations. Forearm blood flow (FBF), which examines NO mediated vasodilation, was measured after subjects consumed 5 days of a low sodium diet (10 mmol/d) or 5 days of a high sodium diet (400 mmol/d). When the enzyme eNOS was blocked by L-NMME after administration of acetylcholine, there was a much greater decrease in vasodilatory ability in males than females. This study found that FBF in all subjects was greater in the low sodium diet group compared to the high sodium diet group; further examination revealed that the male FBF was more sensitive to the different diets. This data suggests that NO production may be altered by dietary sodium intake in men to a greater extent than women, which supports our results showing male endothelial function seemed to respond to a greater extent than the two females in our study.

We measured endothelium independent dilation in one subject, and were unable to assess any differences from the baseline to the end of the study. Previous dietary sodium intervention studies have shown no differences in endothelium independent dilation between high sodium and low sodium conditions (DuPont et al., 2012).

In conclusion, we saw no improvements in endothelial function after 30 days of following a low sodium diet, although males tended to show improvements in endothelial function. While these results may suggest an enhanced sensitivity to sodium reductions in men compared with women, our subject numbers are too small to show any significant differences. A longer-term study period with a greater number of subjects may show improvements in endothelial function.

4.3 Arterial Stiffness and Distensibility

Arterial stiffness as assessed by PWV, and wave reflection as assessed by AIx did not significantly differ over time. Our values for PWV were 6.28 ± 0.57 m/s at baseline and 7.8 ± 1.5 m/s at day 30, which are consistent with other studies (Edwards et al., 2008; Jablonski et al., 2013a; Todd et al., 2010). Our values for AIx at baseline were $-6.3 \pm 2.23\%$ and at day 30 were $-6.95 \pm 2.46\%$, which is also consistent with published work (Edwards et al., 2008). A study by Todd et al., (2010) reported PWV values similar to ours, in the 7.29-7.84 m/s range and saw a significant increase in PWV after 4 weeks of salt loading. However, in contrast to this study, we saw no changes in PWV with reducing sodium intake. It may be that subjects respond more quickly to increases in salt loading resulting in increases in PWV as opposed to reduction. Also, this study compared PWV values during a prescribed low sodium diet (60 mmol/d) to a high sodium diet (150-200 mmol/d), rather than subjects' habitual diet. In contrast, a study by Jablonski et al., (2013b) showed that aortic PWV was decreased by 17% after consuming five weeks of a low sodium diet compared to a normal sodium diet. The timeframe of this study was similar to ours, but included a different population of older (51-72 yrs), pre-hypertensive, overweight men and women. It is likely that large artery remodeling takes longer than 30 days, varies in different populations, and thus any changes in PWV sooner are unlikely.

A study by Dickinson et al., (2009) found no changes in PWV or AIx after 2 weeks of a low salt diet (50 mmol/d) compared to a usual salt diet (150 mmol/d). By choosing a period of thirty days, we hoped to see changes in PWV and AIx in habitually high sodium consumers as our intervention was longer. Yet in another study by Dickinson et al., (2014b) they found no changes in PWV or AIx after 6 weeks of a reduced salt diet (6g/d salt) compared to a usual salt diet (9g/d salt). Other studies

have shown inconsistent results in improvements in PWV and AIx after a sodium reduction (Gijssbers et al., 2015). Improvements in PWV have been seen in a period of 6 weeks after a 55mmol/d sodium reduction, but this occurred in a group of older, mildly hypertensive African Americans. We did not see any changes in blood pressure in this study, which could have contributed to the lack of improvements in PWV or AIx.

Acutely, a high sodium meal (65mmol Na⁺) has been shown to increase AIx by 2% in healthy, normotensive adults compared to a low sodium meal (5mmol Na⁺) (Dickinson et al., 2014a). A study by Dickinson et al., (2009) also found that following a low sodium diet for 2 weeks did not improve AIx in a group of overweight and obese adults, possibly due to a short intervention. However, our chronic intervention of following a low sodium diet did not significantly change AIx. Other studies have shown inconsistent results in improvements of AIx and PWV, possibly due to subject characteristic variability, and interventions. For example, while we saw no improvement in AIx after 30 days, however a study by Gates et al., (2004) found an improvement in AIx and arterial stiffness after a reduction to 60 mmol/d for 4 weeks in older, hypertensive adults. We had a similar timeframe, but our study participants were relatively younger, and free of hypertension. Another study by Seals et al., (2001) demonstrated improvements in AIx and PWV in healthy post-menopausal women, but interventions included dietary sodium restriction in addition to an exercise intervention for three months.

In summary, these studies demonstrated a detrimental effect of sodium loading on arterial stiffness and wave reflection while our salt reduction approach did not alter

indices of arterial stiffness. It may be possible that longer periods of sodium restriction are warranted to see changes in a younger, healthy population free of hypertension.

4.4 Microvascular Function

Microvascular function was assessed by the cutaneous vasodilation response to hyperemia. Microvascular dysfunction has been demonstrated previously in response to salt loading independent of changes in blood pressure (Greaney et al., 2012). While there were no changes in blood pressure in our subjects during the study period, we saw no significant change in microvascular function. We were able to collect data on 7 of 8 subjects as we lost microvascular data on one subject at day 30 due to skin irritation. It should be noted that we do have missing data points with our Ascorbic Acid site due to a heater malfunction that has limited our ability to draw conclusions on our data.

Absolute maximal CVC did not differ between baseline and day 30 across the two different sites, consistent with other studies (DuPont 2011; DuPont 2014; Greaney et al., 2012). There was a significant difference in baseline %CVCmax at the Ringer site between baseline and day 30, and at baseline between the Ringers and Ascorbic acid site. However, when the data was pooled together, there was no difference in baseline %CVCmax.

In contrast to other studies, there was no difference in the NO-mediated plateau %CVCmax between baseline and day 30 at the Ringers site or in comparison to Ascorbic acid. Greaney et al., (2012) demonstrated a reduction in the plateau %CVCmax at the Ringer site in subjects consuming a high sodium diet compared to a low sodium diet. It is possible that we did not see any differences due to the high variability of sodium intakes among subjects' habitual intake, while the Greaney study

was a controlled feeding study in which subjects consumed 350 mmol/d compared to 20 mmol/d Na⁺ for a week each. This study also demonstrated that infusion of ascorbic acid restored the NO-mediated plateau during the high sodium diet when compared with ringers while we showed a trend towards significance at baseline alone. Again, our limited subjects likely contribute to the lack of significance.

In conclusion, these data suggest that a sodium reduction for 30 days may not be long enough to result in changes microvascular function and more likely, we need additional subjects to completely answer this question.

4.5 Anthropometric and Biochemical Parameters

Overall, subjects weight and body fat remained stable over the 30-day period. Our goal was for weight to remain stable in all subjects, as weight loss and change in body fat percent can affect vascular function (Pierce et al., 2008). Upon closer examination of subjects' anthropometrics, one subject had a ~5% weight reduction over the 30-day period while all other subject's weight remained stable.

The average baseline urinary sodium excretion was 156.1 ± 20.3 mmol/L (range 65.5-263.1 mmol/24hr), and decreased at the midpoint visit (85.5 ± 15.9 mmol/L) and at day 30 (99 ± 12.2 mmol/L). This baseline sodium intake equates to ~3,590 mg/d Na⁺ (6,049-1,495 mg Na⁺), and on average is less than what we our goal was ($\geq 5,000$ mg/d). Our three-day diet records did not coincide with the 24-hr urine collection at the baseline-testing visit that likely explains some of the discrepancy. Hence, we were not able to correlate the sodium intake from the dietary recalls with the urinary sodium excretion, as they were not consistently done on the same day. It may be that collecting 24-hr urine and blood pressure was burdensome to our

subjects', and wearing the ambulatory blood pressure monitor and carrying the urine collection container may have had an impact on our subject's usual diet. Enrollment in the study might also have affected subjects' diets, as they were aware of the goal of the study. Finally, it may be that subjects changed their diets during from recruitment to start of the study.

While baseline sodium excretions were not as high as expected, there was a decrease in 24-hr urinary sodium excretion at day 30 when compared to baseline, although not significant; there was however a significant reduction at the midpoint collection compared to baseline. Subject's midpoint dietary recall was also the lowest out of the 5 recalls, averaging 1111 ± 253 mg/d. This dip in sodium intake and excretion may be due to an over restriction in sodium intake within the first 2 weeks of the intervention, suggesting subjects may have struggled to maintain stable sodium intakes for the entire 30 days.

Other dietary counseling studies have utilized 24 hr collections to assess urinary electrolytes, osmolality, hormones and mean arterial pressure. A study by Dickinson et al., (2009) used collection of 24-hr urine at the end of each 2-week study period to assess sodium and potassium excretion as well as compliance to the prescribed diet. This is similar to our study as we had our subjects collect 24-hr urines, but we did so three times during the study intervention-at baseline, midpoint, and day 30. Another study by Dickinson et al., (2014b) used a combination of multiple 24-hr urine collections and 24-hr blood pressure collections during a 12-week long study period. If we used multiple 24-hr urine collections at baseline, we could have potentially gotten a more accurate picture of habitual sodium intake. Future studies

could consider using multiple 24-hr urine collections in conjunction with a three-day food log at baseline to assess habitual sodium intake.

Subjects' urinary potassium excretion did tend to increase at day 30 compared to baseline; upon further analysis we see that there was two subjects who were primarily responsible for this increase. Analysis of the 24-hr recalls indicates that potassium intake did not significantly differ over the 30-day intervention period compared to baseline. Our goal was to keep habitual potassium intake constant with only changing sodium intake.

In conclusion, there was a decrease in urinary sodium excretion that was consistent with the decrease in sodium intake, however baseline sodium excretion values were not as high as expected based on the habitual dietary data.

4.6 Limitations

There are several limitations in this study. First, this was a pilot study, and therefore we had a small number of subjects (n=8), with a wide age range (22-58 years). Hence, we are underpowered to fully answer our questions. This study included a total of 6 males and two postmenopausal females. Therefore, the results of this study may not be applicable to young females.

We also have to consider the accuracy of self-reported dietary intakes. While the 24-hr recall using the Automated Multiple Pass Method has been validated and is a widely used tool, there exists a chance for subject and interviewer error. Habitual intake of sodium was assessed from a three-day food log during the screening, and hence, their sodium intake could have decreased by the baseline visit. Further, we did not collect a 24 hr urine when subjects recorded their 3 day food record which is how we classified subjects as high sodium consumers nor did we collect a 24-hr recall

during their baseline testing visit. Additionally, we did not retrospectively examine subjects' sodium intake and therefore, the length of increased sodium intake could have varied from subject to subject resulting in different responses to the intervention.

Finally, we are missing several data points from the microvascular data due to the challenges of this technique.

4.7 Future Directions

Future research in this group of individuals would warrant a more diverse population. A longer-term intervention may also be warranted. Confirmation of habitually high levels of sodium may be done using different methods other than a three day food log; perhaps a three day food log in conjunction with multiple 24-hr urine collections would more accurately reflect habitual sodium intake.

While we did target sodium specifically in this population, there were inevitably changes to other aspects of the diet. Urinary potassium excretion increased at the end of the 30-day study period, indicating that subjects were consuming more potassium at the end of the study than at the beginning. This is possibly due to making better choices and choosing healthier options.

4.8 Conclusions

In conclusion, dietary counseling to lower sodium intake to 2g/day for thirty days was not able to improve endothelial function or arterial stiffness in a group of generally healthy, non-obese habitually high sodium consumers (≥ 5 g/day) free of hypertension. Dietary counseling was effective at lowering sodium intake as sodium intake was significantly reduced at each of the five dietary recalls compared to

baseline. Longer term interventions in a larger sample of subjects should be explored in future studies.

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Appendix A

MEDICAL HISTORY QUESTIONNAIRE



540 S College Ave, Suite 130
Newark, DE 19713
Phone: 302-831-3195; Fax: 302-831-3193
Email: nm-hc@udel.edu

Research Participant Medical Questionnaire

I. Personal Information

First Name: _____ Middle Name: _____

Last Name: _____

Home Street Address: _____

City, State, Zip: _____

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

Gender: Male Female Age: _____

Marital Status: Married Single Divorced Widowed Legally Separated Other

Race: Caucasian Black Hispanic Asian Native American Pacific Islander

Asian Pacific American Alaskan Native Black-Non Hispanic

White-Non Hispanic Other (supply name) _____

Personal ph#: _____ Work ph# _____

Email Address: _____

Emergency Contact

First Name: _____ Personal ph#: _____

Last Name: _____ Work ph#: _____

How are you related to the Emergency Contact?

You are the: Spouse Parent Son/Daughter Grandchild Niece/Nephew Aunt/Uncle Employee

II. Personal Physician Contact Information

Do you have a personal physician: Yes No

Physician's Full Name _____

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

Physician's Full Name _____

III. I attest that the personal and medical information provided is correct to the best of my knowledge.	
Participants Signature: X	Date:
Reviewed by Clinician's signature:	Date:

IV. Medical Information															
In what research study are you participating?	<input type="checkbox"/> EPR <input type="checkbox"/> Dietary Salt <input type="checkbox"/> Women's Skin Blood Flow <input type="checkbox"/> FastFES <input type="checkbox"/> FAM <input type="checkbox"/> Sodium/Potassium Uptake <input type="checkbox"/> Salt Sensitivity <input type="checkbox"/> Potassium Study <input type="checkbox"/> Exercise Training Study <input type="checkbox"/> Cocoa Study														
Check any of the medical conditions listed that you have been diagnosed with:	<input type="checkbox"/> High Blood Pressure <input type="checkbox"/> Heart Disease <input type="checkbox"/> High Cholesterol <input type="checkbox"/> Kidney Disease	<input type="checkbox"/> Asthma <input type="checkbox"/> Emphysema <input type="checkbox"/> Cancer <input type="checkbox"/> Anemia	<input type="checkbox"/> Diabetes <input type="checkbox"/> Stroke <input type="checkbox"/> Blood Clots												
List any other medical diagnosis you have:															
Have you been hospitalized for any significant injury or illness: <input type="checkbox"/> Yes <input type="checkbox"/> No															
If yes list reason and dates:															
Check any of the medical conditions listed that either your Father or Mother have been diagnosed with:	<table border="0"> <tr> <td>F</td> <td>M</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> </table> <input type="checkbox"/> High Blood Pressure <input type="checkbox"/> Heart Disease <input type="checkbox"/> High Cholesterol <input type="checkbox"/> Kidney Disease	F	M	<input type="checkbox"/>	<input type="checkbox"/>	<table border="0"> <tr> <td>F</td> <td>M</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> </table> <input type="checkbox"/> Asthma <input type="checkbox"/> Emphysema <input type="checkbox"/> Cancer <input type="checkbox"/> Anemia	F	M	<input type="checkbox"/>	<input type="checkbox"/>	<table border="0"> <tr> <td>F</td> <td>M</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> </table> <input type="checkbox"/> Diabetes <input type="checkbox"/> Stroke <input type="checkbox"/> Blood Clots	F	M	<input type="checkbox"/>	<input type="checkbox"/>
F	M														
<input type="checkbox"/>	<input type="checkbox"/>														
F	M														
<input type="checkbox"/>	<input type="checkbox"/>														
F	M														
<input type="checkbox"/>	<input type="checkbox"/>														
If you are currently taking any prescription medicine, over-the-counter medicine, vitamins, herbs, nutritional supplements or birth control pills, please list the medication name, dosage and frequency taken below															
1.	5.														
2.	6.														
3.	7.														
4.	8.														
Are you ALLERGIC to any medication, food or latex?	<input type="checkbox"/> Yes <input type="checkbox"/> No	If yes what?	Type of reaction:												
Do you smoke?	<input type="checkbox"/> Yes <input type="checkbox"/> No	If yes how much?	How many years?												
Did you ever smoke?	<input type="checkbox"/> Yes <input type="checkbox"/> No	If yes, quit date?	# Yrs. smoked?												
Do you drink alcohol?	<input type="checkbox"/> Yes <input type="checkbox"/> No	If yes how much?	How many years?												
Do you drink caffeinated drinks	<input type="checkbox"/> Yes <input type="checkbox"/> No	If yes how much?	Type: <input type="checkbox"/> Coffee <input type="checkbox"/> Tea <input type="checkbox"/> Soda												
Do you normally eat a balanced diet	<input type="checkbox"/> Yes <input type="checkbox"/> No	Meals per day?	Snacks per day?												

Do you exercise on a regular basis?	<input type="checkbox"/> Yes	<input type="checkbox"/> No	Days per week?	Type of:
-------------------------------------	------------------------------	-----------------------------	----------------	----------

Have you had any of the following tests? If yes, include last year you had the test.				
<input type="checkbox"/> EKG	Year	<input type="checkbox"/> Stress Test	Year	<input type="checkbox"/> Mammogram
<input type="checkbox"/> Colonoscopy	Year			

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

Appendix B

FOOD RECORD INSTRUCTIONS

INSTRUCTIONS FOR KEEPING YOUR 3-DAY DIET RECORD

The following instructions will help you to complete a three-day food intake log successfully. You will be provided a booklet entitled *USDA Food Models for Estimating Portions* to help estimate the portions of foods you have consumed. You will record this data on your three-day food intake log, an example of which is provided below. 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 recommendations on how to most accurately record your food and beverage consumption.

Please keep in mind:

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. 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.

Meal/ Time of Day	Food/Drink (specify brand name or restaurant, if applicable)	Describe Preparation Methods	Choose One			# of SVGS
			Model # and to What Line	OR	Pre- Packaged Item	
Breakf ast 8:30 am	Coffee Honey Nut Cheerios Soymilk (Silk)		MG3 Line A			1
			B4 Line B			1
			B4 Line D			1
Snack 11:00 am	Nature Valley Granola Bar Diet Coke				1 package	
					1-8oz can	2

Meal/Time of Day: Please indicate whether you ate breakfast, lunch, snack, dinner, or dessert; also include the time of day the food was eaten. If you grazed, you may indicate this and write down a time span.

Food/Drink: This is where the item consumed is written down. If you eat in a 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. Include all the beverages you consume, including alcohol and water.

Describe Preparation Methods: Describe how the food was prepared (fried, boiled, baked, etc) and how it was served (with cream sauce, Italian dressing, etc). This is useful when meals are prepared at home; homemade recipes; additives or meal toppings or anything else included in the meal. Be sure to include all sauces, gravies, dressings, cream and sugar for coffee, etc., as these items contribute to your total calorie intake.

Estimate as closely as you can the portion size you consumed. Some examples of typical portion sizes can be found at the bottom of the page. **Choose one of the following**

Model Number and to What Line: Please use the USDA Food Models for Estimating Portions booklet provided to you to estimate portion sizes. You will indicate what Model Number and to what corresponding line you consumed the item. Use this column if you want to easily estimate items that are not pre-packaged or easily measured. Examples include glasses, mugs, bowls, plate sizes, etc.

OR

If Pre-Packaged Item: If you consume an item that is prepackaged with the serving size, please use this column. Indicate the brand of packaged item.

of SVGS: Indicate if you had more than one serving of the item; ex: 2 apples, 2 bowls of chili, etc.

Estimating Portion Sizes

Fruits & Vegetables

- 1 c of fruit or vegetables = a baseball
- 1 medium sized fruit = a tennis ball
- $\frac{1}{4}$ c dried fruit = a golf ball
- 2-inch slice of melon = width of 3 fingers

- Medium potato = size of a computer mouse

Meats, nuts, & other protein rich foods

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

Dairy

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

Breads & 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 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 (i.e. Jellybeans): about one handful
- 4 small cookies (like vanilla wafers) = four checkers or poker chips

Appendix D

INSTRUCTIONS FOR PARTICIPANTS: 24-HR URINE COLLECTION AND BLOOD PRESSURE MONITOR

Instructions for Participants: 24-hr urine and blood pressure collection

24-hr urine collection

You will be given an orange 2-L urine collection container where you will collect your urine for 24-hours following testing visits 2 and 5. This container will be given to you in a clear plastic bag, inside of a black backpack to make transportation easiest. There will be a paper in which you will indicate the time you last emptied your bladder on the day of testing- this is the start of the 24-hr urine collection. You will start collecting urine for the following 24-hrs and cease urine collection the same time of the following day. Write down the time of the last urine collection for the following day.

For example: You last emptied your bladder at 7:30am in a toilet. Your testing visit 2 is 8am-12pm at STAR Campus. You will collect your urine the rest of the day using the orange container provided, and when you wake up the next morning, collect your urine one last time (around 7:30am).

24-hr blood pressure cuff

A 24-hr blood pressure monitor will be placed on your arm following testing visits 2 and 5. This blood pressure cuff will automatically take readings 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. Please do not remove the cuff for extended periods of time (i.e. to shower); if you are uncomfortable and need to make a slight adjustment, that is fine. Once you feel the cuff start to inflate, try to sit down relax your arm if possible, so it will get a good reading. If the monitor did not get a good reading, it will re-inflate to capture another reading. The monitor records and saves each blood pressure measurement automatically. The blood pressure cuff and urine collection will go hand in hand, so when you collect your last urine, you may turn off the 24-hr blood pressure cuff and remove it from your arm.

Please return the 24-hr urine collection and 24-hr blood pressure cuff to the STAR Campus at visits 3 and 6.

***Remember:** no physical activity, caffeine, or alcohol intake while collecting on these days!

If you have any questions, comments, or concerns, please contact one of the following.

○ Karen Solecki, RD

609-744-2613 (cell)

karensolecki@gmail.com (email)

Appendix E

LOW SODIUM HANDOUT

Name: _____

Date: _____

Nutrition Facts	
Serving Size 1/2 cup (57g)	
Servings Per Container 15	
Amount Per Serving	
Calories 240	Calories from Fat 70
% Daily Value*	
Total Fat 8g	12%
Saturated Fat 0.5g	1%
Trans Fat 0g	
Cholesterol 0mg	0%
Sodium 90mg	4%
Potassium 250mg	7%
Total Carbohydrate 37g	12%
Dietary Fiber 4g	8%
Sugars 18g	
Protein 5g	
Vitamin A 0%	Vitamin C 0%
Calcium 4%	Iron 10%
<small>*Percent Daily Values are based on a diet of other people's secrets. Your daily values may be higher or lower depending on your calorie needs.</small>	
Calories 2,000 2,500	
Total Fat	Less Than 65g 65g
Saturated Fat	Less Than 25g 25g
Cholesterol	Less Than 300mg 300 mg
Sodium	Less Than 2,400mg 2,400mg
Potassium	2,500 mg 3,000 mg
Total Carbohydrate	300g 370g
Dietary Fiber	25g 35g
<small>Calories per gram: Fat 9 • Carbohydrate 4 • Protein 4</small>	

Look for the following on the Nutrition Facts Label:

- **Serving size:** all of the information on the label about calories and nutrients is for 1 serving; if you eat more than 1 serving, you get more calories and nutrients
- **Calories:** Choose foods that help you get the nutrients you need without going over your daily calorie goal. Too many calories leads to weight gain
- **Total fat:** Choose foods with less than 5g total fat/serving. Try to pick foods with heart healthy fats (monounsaturated and polyunsaturated fats)
- **Saturated and trans fat:** choose foods with less than 3g/serving saturated and *trans* fat. Read ingredients, if it contains partially hydrogenated oils, ~~then~~ it has trans fat.
- **Sodium:** Look for foods that are low in sodium. Each day, maintain a 2,000mg (2g)/day sodium diet.
- **Dietary fiber:** Aim to get 25-30 g dietary fiber each day. To meet this goal, try to eat foods with at least 5g fiber/day

The target is to keep your daily sodium intake at **2,000 mg** per day. By following some of the tips that are listed below, as well as making some of the recommendations made for food swaps created with the Dietitian (see reverse side of page), you will be able to achieve this level. Remember to always read labels to learn the sodium content per serving!

Try to avoid these items:

- Table salt: ¼ of a teaspoon has ~600mg of sodium
- Condiments, sauces and seasonings
 - Ketchup, mustard, salad dressings, bouillon cubes
 - Sauces- Worcestershire, barbeque, pizza, chili, steak, soy, horseradish sauce
 - Celery salt, garlic salt, onion salt; it is OKAY to have garlic or onion powder
 - Pickles, olives
 - Read the labels for seasonings- some have salt (lemon pepper)
- Processed foods
 - Frozen foods- dinners, entrees, vegetables with sauces often contain high amounts of sodium
 - Canned foods- soup, stews, sauces, gravy, vegetables, beans
 - Snack foods
 - Meats and cheeses
 - Deli or lunch meats- bologna, ham, turkey, roast beef, **etc**
 - Cured or smoked meats- corned beef, sausage of any kind (Italian, patty, wiener, hot dog, kielbasa)
 - Canned meats- potted meats, spread, Spam, Vienna sausage
 - Cheeses- read labels (avoid any containing >140 mg sodium/serving) like American, **velossta**, **cheez** whiz

Soy Sauce Substitute

2 tbsp. reduced sodium beef broth
1 tbsp. red wine vinegar (0g sodium)
1 tsp balsamic vinegar (0g sodium)
2 tsp molasses (14g sodium)
1 tsp sesame oil (0g sodium)
1/8 tsp garlic powder (0g sodium)
Black pepper to taste (0g sodium)
¼ cup boiling water (0g sodium)

Combine all the ingredients. At this point, you can either use the sauce as it is, leaving for an hour to give the flavors a chance to blend, or boil the liquid until it is reduced by half (for a thicker, richer sauce). Store in a sealed container in the refrigerator and use within 3-4 days. Yields about 1/3 cup.

*You could also use a sodium-free beef broth with 0 sodium available from healthyheartmarket.com

Internet source of excellent low-sodium recipes: lowsodiumcooking.com
Internet source of low-sodium foods: healthyheartmarket.com

Barbeque Sauce

½ cup low-sodium ketchup
½ cup vinegar
½ cup honey
¼ cup molasses
1 tbsp. chili powder
1 tbsp onion powder
½ tsp garlic powder
1 tbsp dry mustard
¼ tsp cayenne pepper

Amount per serving	
Calories	94
Calories from fat	3
Total Fat (g)	0
Saturated Fat (g)	0
Polyunsaturated Fat (g)	0
Monounsaturated Fat (g)	0
Cholesterol (mg)	0
Sodium (mg)	7
Potassium (mg)	224
Carbohydrates (g)	25
Dietary Fiber (g)	0.5
Protein (g)	1

Combine all ingredients and mix well. Store in a covered jar in the refrigerator. 10 servings.

Appendix F

SCRIPT FOR 24-HR RECALL

Script for 24-hr recall (either in-person or over the telephone)

“We have an appointment scheduled for today for your 24-hr Dietary Intake Interview. Do you have the food model booklet available?”

Wait for subject to take out food model booklet..

“We will be going through a list of all the food and drinks consumed in the past 24 hours to get an idea of your dietary intake, specifically sodium. We will first go through a general list of items consumed starting when you first woke up in the morning. Then we will go through and specify time of day these foods were eaten, as well as quantity, method of preparation.”

Step1: Quick List

“The first thing we are going to do is gather a list of everything you ate yesterday from midnight to midnight. Then we will go through and gather information on the specific time, occasion and quantity of when these foods were consumed.”

When finished the Quick List..

“Alright, anything else yesterday to eat or drink?”

If help is needed: prompt subject on what they did yesterday..

Step 2: Forgotten foods

“So now what we are going to do is go through our *forgotten foods list*. Did you consume any coffee, tea, soft drinks, milk, juice? How about any beer, wine, or alcoholic beverages? How about any cookies, candies, sweets? How about any chips, crackers? Any fruit/vegetables, cheese? Any tortillas, bread other than what you already mentioned?”

“Anything as small as a piece of chewing gum or a mint?”

Step 3: Time Occasion

“Now we will look at the time foods were consumed.”

Go through each food and gather the time consumed and what occasion this is considered (8am- breakfast, 1pm- lunch, 6pm-dinner, 8pm-snack?)

Step 4: Detail Cycle

“Now we will go through this list to gain the quantities of food using the food model booklet.

Did you have anything to eat between midnight yesterday and your breakfast?” (repeat this for each meal & snack)

Amounts using FMEP 2007

Glasses: pages 3 or 4

Wine Glasses: page 5

Mug: Page 6

Bowls: pages 7 or 8

Small Mounds: page 9

Large Mounds: pages 10-14

Plate: page 16

Go over the final components of the meal at the end before moving on to next occasion:

- Preparation Methods: at home or away from home (fast food)
- Brand (if possible)
- What was it made with? Salt? Butter? Did you bake, fry, broil, deep fry?
- Did you consume/drink all of the food/beverage?

Step 5: Final Probe (small like gum/mint, water?)

Is there anything else you can think of?

In reference to salt intake, did you add any salt at the table? Add salt while cooking? Regular salt or salt substitute?

Extra Notes:

Appendix G

IRB HUMAN SUBJECTS APPROVAL



RESEARCH OFFICE

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

DATE: April 16, 2015

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

STUDY TITLE: [594590-5] A Pilot study on the Role of Dietary Counseling on Salt Consumption and Vascular Function

SUBMISSION TYPE: Continuing Review/Progress Report

ACTION: APPROVED
APPROVAL DATE: April 15, 2015
EXPIRATION DATE: April 15, 2016
REVIEW TYPE: Full Committee Review

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 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 Nicole Farnese-McFarlane at (302) 831-1119 or nicolefm@udel.edu. Please include your study title and reference number in all correspondence with this office.

Appendix H

PHYSICAL ACTIVITY QUESTIONNAIRE

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health-related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an *International Physical Activity Prevalence Study* is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. *Research Quarterly for Exercise and Sport*, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

Yes

No →

Skip to PART 2: TRANSPORTATION

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

____ days per week

No vigorous job-related physical activity →

Skip to question 4

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

____ hours per day

____ minutes per day

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

____ days per week

No moderate job-related physical activity →

Skip to question 6

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?
- _____ hours per day
 _____ minutes per day
6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.
- _____ days per week
- No job-related walking → **Skip to PART 2: TRANSPORTATION**
7. How much time did you usually spend on one of those days **walking** as part of your work?
- _____ hours per day
 _____ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?
- _____ days per week
- No traveling in a motor vehicle → **Skip to question 10**
9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?
- _____ hours per day
 _____ minutes per day

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?
- _____ days per week
- No bicycling from place to place → **Skip to question 12**

11. How much time did you usually spend on one of those days to bicycle from place to place?
- ____ hours per day
 ____ minutes per day
12. During the last 7 days, on how many days did you walk for at least 10 minutes at a time to go from place to place?
- ____ days per week
- No walking from place to place → *Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY*
13. How much time did you usually spend on one of those days walking from place to place?
- ____ hours per day
 ____ minutes per day

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the last 7 days in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, chopping wood, shoveling snow, or digging in the garden or yard?
- ____ days per week
- No vigorous activity in garden or yard → *Skip to question 16*
15. How much time did you usually spend on one of those days doing vigorous physical activities in the garden or yard?
- ____ hours per day
 ____ minutes per day
16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, sweeping, washing windows, and raking in the garden or yard?
- ____ days per week
- No moderate activity in garden or yard → *Skip to question 18*

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis in **your leisure time**?

_____ **days per week**

No moderate activity in leisure time



Skip to PART 5: TIME SPENT SITTING

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

Appendix I

RECRUITMENT FLYER



Are you interested in learning how to change your diet to improve your heart health?

The Department of Behavioral Health and Nutrition and Department of Kinesiology and Applied Physiology are conducting a study looking at the effects of dietary counseling on heart health in people who normally consume increased levels of salt



You may be eligible to participate if:

- You have participated in the UD Salt Study
- You are between the ages of 22-65
- You have normal blood pressure

Please contact Karen Solecki by phone at (609) 744-2613 or email by ksolecki@udel.edu