Comparison of High-Nitrate versus Low-Nitrate Diets on Cardiovascular Health in Post-Menopausal Women

by

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ABSTRACT

Background**.** Despite research aimed at understanding the mechanisms of essential hypertension, instances of this condition continue to rise. Recent findings indicate that the administration of dietary nitrates, in the form of beetroot juice and other nitrate-rich vegetables, may offer anti-hypertensive effects in various study populations.

Objective. This randomized, placebo-controlled, crossover trial sought to compare the effects of high-nitrate vegetable salads to the effects of low-nitrate canned vegetables on plasma nitrate/nitrite concentration, peripheral and central-aortic systolic and diastolic blood pressures, pulse wave velocity, and flow-mediated dilation.

Methods**.** Healthy, post-menopausal women (n=5; 80% Caucasian; 52.6 ± 5.7 years) with mildly elevated blood pressure (mean blood pressure ≥ 115/70 mm Hg and < 140/80 mm Hg) were randomly assigned to ingest a fresh, high-nitrate vegetable salad or a low-nitrate vegetable medley, twice per day, for a total of 10 consecutive days. Given the crossover design of the trial, participants observed a two to three week washout period followed by reassignment to the opposite condition. Findings were considered significant at a p-value < 0.05, and Wilcoxon Signed-Rank tests compared mean differences between conditions.

Results**.** Plasma nitrate/nitrite concentration was significantly higher following consumption of the high-nitrate versus the low-nitrate condition ($p = 0.043$). Conversely, the differences in peripheral systolic and diastolic blood pressures were not statistically significant ($p = 0.345$ and $p = 0.684$ for systolic and diastolic pressures, respectively) nor were the differences in central-aortic systolic and diastolic blood pressures statistically significant ($p = 0.225$ and $p = 0.465$ for systolic and diastolic pressures, respectively).

Similarly, when comparing the effects of the high-nitrate condition to the low-nitrate condition, the difference in pulse wave velocity was not statistically significant ($p = 0.465$). Finally, flow-mediated dilation tended to improve following consumption of the high nitrate condition ($p = 0.080$).

Conclusion. Twice daily consumption of a fresh, high-nitrate vegetable salad significantly increased plasma nitrate/nitrite concentration. Although the trial was underpowered, there was a trend for improved flow-mediated dilation. Finally, twice daily consumption of a fresh, high-nitrate vegetable salad did not significantly lower peripheral or central-aortic systolic or diastolic blood pressures or pulse wave velocity.

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DEDICATION

I dedicate this thesis project to my family. Thank you for your unwavering love, support, and encouragement.

To my darling – thank you for continuing to believe in me. I love you.

TABLE OF CONTENTS

Page

CHAPTER Page

LIST OF TABLES

Table Page

CHAPTER 1

INTRODUCTION

Problem

Hypertension, or high blood pressure, is a major global health concern and in the U.S. alone, nearly one out of every three adults struggle with this largely preventable condition.[1] Further, hypertension is linked to almost 1,000 deaths per day,[1] and the CDC predicts that hypertension-related healthcare expenditures will exceed 270 billion dollars by 2030.[2] Additionally, hypertension has been shown to increase the likelihood of chronic conditions including stroke, heart attack, coronary heart disease, heart failure, and kidney failure.[3-5] Previous findings suggest that the prevalence of hypertension increases from early adulthood well into older adulthood.[1] Next, research indicates that, on the basis of sex, there are notable differences between the onset of the condition. Specifically, when compared to similarly aged women, hypertension is more common in men 45 years of age or younger. Conversely, when compared with similarly aged men, the disease is reportedly more common in women 65 years of age or older.[1,6]

To date, researchers have focused their efforts on identifying attainable strategies to prevent the development of hypertension by modifying certain lifestyle behaviors. One such behavior believed to be linked to hypertension is dietary intake. Numerous studies have focused on eliminating certain foods, which are believed to be responsible for increasing blood pressure, from one's diet (e.g., foods high in sodium), while other studies have focused on consuming functional foods that may be responsible for lowering blood pressure.[7-9]

One functional food believed to lower blood pressure is beetroot juice (*Beta vulgaris*). However, studies that have utilized this nitrate-rich food source have been somewhat inconsistent, and have included a variety of study populations which may make interpretation of the findings somewhat difficult.[9-14] Further, few studies have collectively assessed the effect of nitrate-rich whole foods on plasma nitrate/nitrite concentration, peripheral and central-aortic systolic and diastolic blood pressures, pulse wave velocity, and flow-mediated dilation.[15,16]

Through the entero-salivary nitrate-nitrite-nitric oxide pathway, it has been shown that dietary nitrate is effective for improving endothelial function, and for lowering blood pressure in healthy adults.[10,17] Given the high prevalence of, and economic burden associated with hypertension in the U.S.,[1] it is vital to understand how nitrate-rich whole foods can be incorporated into an overall healthful lifestyle to combat this disease. Thus, findings from this study may be beneficial for designing appropriate lifestyle interventions to improve cardiovascular health in otherwise healthy, post-menopausal women (40-70 years old).

Purpose of the Study

The primary intent of this randomized, placebo-controlled, crossover trial was to compare the effects of a nitrate-rich vegetable salad to a low-nitrate vegetable medley, consumed twice per day, on plasma nitrate/nitrite concentration, peripheral and central-aortic systolic and diastolic blood pressures, pulse wave velocity, and flow-mediated dilation in healthy, post-menopausal women residing in Phoenix, Arizona. Beyond these parameters, height (in cm) and weight (in kg) were also assessed.

Research Aims and Hypotheses

Study Aims: To compare the effects of a fresh nitrate-rich vegetable salad, consumed twice per day, to a canned low-nitrate vegetable medley, consumed twice per day on (1) plasma nitrate/nitrite concentration, (2) peripheral systolic and diastolic blood pressures, (3) central-aortic systolic and diastolic blood pressures, (4) pulse wave velocity, and (4) flow-mediated dilation in healthy, post-menopausal women residing in Phoenix, Arizona.

Research Question 1: Is there a relationship between the consumption of a fresh nitrate-rich vegetable salad, consumed twice per day, on plasma nitrates/nitrite concentration in healthy, post-menopausal women?

> **H_a**: Subjects will have a higher plasma nitrates/nitrite concentration following consumption of a fresh, nitrate-rich vegetable salad versus a canned vegetable medley twice per day.

Research Question 2: Is there a relationship between the consumption of a fresh nitrate-rich vegetable salad, consumed twice per day, on peripheral systolic and diastolic blood pressures in healthy, post-menopausal women?

> **H_a**: Subjects will have lower peripheral systolic and diastolic blood pressure following consumption of a fresh, nitrate-rich vegetable salad versus a canned vegetable medley twice per day.

Research Question 3: Is there a relationship between the consumption of a fresh nitrate-rich vegetable salad, consumed twice per day, on central-aortic systolic and diastolic blood pressure in healthy, post-menopausal women?

H_a: Subjects will have lower central-aortic systolic and diastolic blood pressure following consumption of a fresh, nitrate-rich vegetable salad versus a canned vegetable medley twice per day.

Research Question 4: Is there a relationship between the consumption of a fresh nitrate-rich vegetable salad, consumed twice per day, on pulse wave velocity in healthy, post-menopausal women?

H_a: Subjects will have lower (improved) pulse wave velocity following consumption of a fresh, nitrate-rich vegetable salad versus a canned vegetable medley twice per day.

Research Question 5: Is there a relationship between the consumption of a fresh nitrate-rich vegetable salad, consumed twice per day, on flow-mediated dilation in healthy, post-menopausal women?

H_a: Subjects will have higher (improved) flow-mediated dilation following consumption of a fresh, nitrate-rich vegetable salad versus a canned vegetable medley twice per day.

Definition of Terms

Cardiovascular diseases: disorders (e.g., stroke, coronary heart disease) affecting the heart and blood vessels.

Diastolic blood pressure: pressure (mm Hg) recorded during the period of relaxation between heartbeats.

Flow-mediated dilation: a measure of endothelial function used to evaluate cardiovascular disease risk.

Hypertension: elevated systolic and diastolic blood pressure, (≥ 140/90 mm Hg).

Post-menopausal: a period of at least 12 consecutive months without menstruation.

Pulse wave analysis: a technique used to generate a central waveform to determine central-aortic systolic and diastolic blood pressures.

Pulse wave velocity: a technique used to assess arterial stiffness.

Nitrate (NO_3^-): a nitrogen containing compound found primarily in green, leafy vegetables which may play a role in vasodilation once reduced to nitric oxide.

Nitrite (NO_2^-): a nitrogen containing compound produced after the reduction of nitrates; nitrites may be further reduced to nitric oxide.

Nitric oxide: a signaling molecule that promotes vasodilation of the arteries resulting in lower blood pressure.

Systolic blood pressure: pressure (mm Hg) recorded while the heart is contracted.

Delimitations and Limitations

Delimitations of this study are healthy, post-menopausal women (40-70 years old) regardless of race/ethnicity, weight status, BMI classification, and socioeconomic status with blood pressure levels (≥ 115/70 mm Hg and < 140/80 mm Hg), subjects not taking hypertensive medication, alcoholic beverages, multi-vitamins, anti-histamines, decongestants, and antiseptic/antibacterial mouthwash. Findings from this study may not be applicable to other populations, or geographic regions. As with all research, limitations to this trial were inherent. One such limitation was that results were based on a small sample. Next, it was unknown whether salad greens used in the study were obtained from a single grower which may have led to differences in nitrate content. Yet

another limitation was that the nitrate levels of vegetables used in the study were not measured.

CHAPTER 2
REVIEW OF LITERATURE

Overview

Hypertension is a major global health concern, and in the U.S. alone, one out of three adults are afflicted with this largely preventable disease while another one in three adults have pre-hypertension, a condition in which blood pressure is higher than normal, but below the defined reference range.[1,2,18] In 2013 alone, hypertension was determined to be the primary cause of, or a contributing factor to almost 1,000 deaths per day,[1,2] and the CDC predicts that hypertension-related healthcare expenditures will exceed 270 billion dollars by 2030.[2] Next, hypertension appears to be a modifiable risk factor for other chronic health conditions including stroke, kidney disease, heart attack, and heart failure,[1-4] and there appears to be a strong, positive correlation between blood pressure and these conditions even in situations where blood pressure levels are normal.[4] Additionally, elevated blood pressure appears to occur in early adulthood, and in the absence of adequate and consistent treatment, symptoms of this condition may become apparent in later stages of life.[1] Thus, this review of literature focuses on five overarching goals. First, to briefly define hypertension and the major forms of the condition, to discuss mechanical factors influencing the condition, to explain the role of the nitric oxide pathway on blood pressure, to describe the role of dietary nitrates on blood pressure and other cardiovascular function tests, and finally, to discuss lifestyle factors that may influence the condition.

Hypertension

Hypertension, or high blood pressure, is a condition in which the flow of blood through the arteries is greater than normal.[19,20] Any recording of blood pressure will include both a systolic and a diastolic measurement. Systolic pressure pertains to the pressure against the arterial walls as the heart contracts while diastolic pressure pertains to the pressure against the arterial walls as the heart is at rest or between beats.[19,20] While it is common for blood pressure to fluctuate times throughout the day (i.e., often increasing after exercise or during high stress situations),[19] continuously elevated blood pressure levels may result in severe strain and/or damage to organs such as the heart and kidneys.[20] Generally, a diagnosis of hypertension is made after the mean of two or more systolic blood pressure readings is at or above 140 mm Hg or after the mean of two or more diastolic blood pressure readings is at or above 90 mm Hg, during two or more subsequent visits to a physician.[3] While hypertension may be linked to genetic pre-dispositions including a mutation to the mitochondrially encoded tRNA isoleucine (MT-TI) gene,[3,21,22] environmental, dietary, and lifestyle factors play a significant role in reducing sub-optimal blood pressure levels, thereby attenuating the negative long-term effects of the condition.[3]

Major Forms of Hypertension

Primary and secondary hypertension are the two main forms of the condition; however, greater incidence is seen with primary hypertension.[3,19,21] Primary hypertension occurs gradually, and in many cases, the true cause of this form of the condition is unknown.[3] Conversely, secondary hypertension generally occurs as a result of one or more underlying health conditions such as obstructive sleep apnea, congenital defects

affecting the blood vessels, kidney complications, or chronic drug and/or alcohol use.[21] While secondary hypertension is often resolved once the underlying condition is under control, primary hypertension is a contributing factor to several chronic health conditions that may have devastating long-term health implications.[3,4,19,21-23]

Prevalence of Hypertension

Data from the 2009-2012 National Health and Nutrition Examination Survey (NHANES) indicated that approximately 33% or 80 million U.S. adults (≥ 20 years) have hypertension, and approximately 45% of those individuals reported that their hypertension was uncontrolled.[1] Further, the prevalence of hypertension appears to vary by race, socioeconomic status, and state of residency.[3,24,25] More specifically, the incidence of hypertension is generally higher among African Americans, individuals who report an annual household income of less than $43,000, and states in which greater than 18.7% of residents live below the poverty line.[25]

The Effect of Chronological Age and Sex on Hypertension

With age, the human body undergoes notable cardiovascular and metabolic changes that may negatively impact health and wellbeing. Thus, it is not surprising that older individuals of either sex are at increased risk for the development of hypertension and other co-morbidities.[26,27] In fact, when compared to individuals between 20-34 years of age, 67% of men and 78% of women over the age of 75 have hypertension. Next, women over the age of 65 are generally more likely to develop the condition while hypertension appears to be more common in men 45 years old or younger.[2] Additionally, it is important to note that individuals between 18-39 years of age are generally less likely to seek medical guidance for their condition.[28] Previous research has demonstrated

that, with advanced age, heart rate, pre- and after-load, and total cardiac output (the amount of blood the heart pumps per minute) generally declines both at rest and during physical activity.[27] Likewise, advanced age generally presents with increased stiffening of the myocardium which increases the likelihood of higher than normal diastolic pressure and arterial wall stiffness – factors that may result in elevated arterial pressure.[27]

Another consequence of advanced age and elevated blood pressure are disturbances in cognitive function. For example, increased systolic blood pressure, in otherwise healthy older adults, has been associated with damage to the brain which may negatively impact cognitive performance in older populations.[29-32] A cohort study conducted on over 900 subjects with high blood pressure (aged 65 or older) and no known history of cognitive impairment revealed that the presence of hypertension significantly increased the risk of cognitive impairment specific to language, visual-spatial function, and lapses in memory.[31] Similarly, a cross-sectional trial conducted on over 6,000 subjects (aged 60 and older) from the NHANES III indicated that normal blood pressure was associated with better cognitive performance while the presence of elevated blood pressure, overt hypertension, or even uncontrolled blood pressure was linked to lower levels of cognitive function in subjects 70 years or older.[30]

The Effect of Ovarian Age on Cardiovascular Diseases

In addition to changes observed during the aging process, several age-related ovarian changes occurring in women have been shown to influence blood pressure.[26] For instance, the onset of menopause presents with pronounced changes in body fat distribution – namely, from a gynoid to an android pattern. Additionally, with the loss of estrogen, a largely protective hormone, post-menopausal women commonly experience

reduced glucose tolerance, high concentrations of triglycerides and lipoprotein(a), low concentrations of high density lipoprotein (HDL), and increased vascular inflammation and endothelial dysfunction – factors which have been shown to be especially impactful for blood pressure control in women.[26,33,34] Additionally, findings from a clinical trial indicated that early post-menopausal women undergoing hormone replacement therapy (HRT) saw protective cardiovascular outcomes when compared to women assigned to the control group (oral calcium). Further, subjects assigned to the control group gained significantly more weight, total body fat mass, and an overall increase in trunk, arm and leg fat while subjects in the intervention group developed no significant changes in these parameters, except for a significant increase in leg fat.[33]

Similarly, when compared to men of similar ages, post-menopausal women appeared to have a higher overall risk for cardiovascular diseases.[26] More specifically, findings from the Framingham Heart Study showed that, after a 30-year follow-up, the incidence of cardiovascular diseases in subjects 35-64 years old age were higher in women than in men.[35] Next, after adjustments were made for age, BMI, and tobacco use, diabetic and hypertensive women had greater mortality-related cardiovascular disease risk than men.[26,36] More alarmingly, in the presence of both diabetes and hypertension, the mortality risk factor increased nearly twofold over that of men.[26,36]

Hypertension and Cardiovascular Diseases

A report released by the World Health Organization states that, among older adults, cardiovascular diseases are the leading cause of death both globally and nationally.[37] In fact, in 2012 alone, approximately 17.5 million U.S. deaths were attributable to various cardiovascular diseases, and globally, coronary heart disease was

responsible for an estimated 7.4 million deaths, while strokes claimed the lives of nearly 6.7 million individuals.[37] To date, hypertension has been implicated as a major contributing factor in the development of these chronic cardiovascular and renal conditions.[3-5] In fact, hypertension is the most significant risk factor for all forms of strokes,[38,39] and epidemiological studies dating back to as early as 1970 suggest that, in instances of controlled hypertension, subjects can expect a dramatic reduction in all-cause stroke mortality.[39,40] On the contrary, when compared with normotensive individuals, those with isolated systolic hypertension tend to experience approximately two to four times as many strokes. Further, a prospective study conducted on over 500 older individuals with hypertension indicated that morning systolic pressure was the strongest predictor of stroke.[41]

Given that the kidneys are crucial in the determination of blood pressure, the association between hypertension and kidney disease remains a matter of considerable interest in the health community.[42] It has been established that elevated blood pressure is deleterious to kidney function.[42,43] Further, it has been shown that systolic, rather than diastolic pressure, is a greater threat to the progression of kidney disease.[43]

Mechanical Factors Affecting Blood Pressure

Within the human body, blood pressure is tightly regulated by several complex mechanisms which involve the heart, kidneys, blood, and blood vessels.[4] Together, these mechanisms work to ensure that blood pressure remains within a designated range. However, prolonged stress and/or assault to these mechanisms may result in long-term structural and functional damage which may negatively impact cardiovascular health and mortality over time.[3]

Endothelial Function

The endothelium is a large, endocrine organ that functions to promote vascular homeostasis.[44,45] The dynamic endothelium lines both lymphatic and blood vessels including capillaries, arteries, and veins.[46] The location of endothelial cells allows for direct contact between lymph, blood, and circulating cells within the body. As such, these highly specialized cells function as protective barriers to control vascular tone and blood fluidity, thereby regulating blood pressure.[46] However, in instances of prolonged elevated blood pressure, endothelial cells become damaged as a result of excessive inflammation. This occurrence, commonly referred to as endothelial dysfunction, results in the eventual development of atherosclerosis, a disease in which plaque (a combination of fatty waste products, cholesterol, and other substances present in the blood) proliferates, remodeling of blood vessels occurs, and synthesis of protective endothelial factors such as nitric oxide diminishes. Collectively, these factors greatly exacerbate the onset and progression of hypertension.[44-46]

Flow-mediated Dilation

Endothelial dysfunction, a noteable risk factor for the development of athlerosclerosis, is present even before overt damage is observed.[46-49] However, traditional measures of endothelial function, such as infusing acetylcoline into target arteries have been invasive and impractical for clinical practice.[48,50] Conversely, since its inception in 1992, the use of flow-mediated dilation, a non-invasive procedure, has become the most commonly utilized technique to measure endothelium-dependent vasodilator function in humans.[45,49,51,52] A study conducted to test the validity of flow-mediated dilation as an indicator of vascular function indicated that the intra-rater variability was 2.9% for brachial

diameter and 1.4% for hyperemic responses.[47] Flow-mediated dilation values are obtained by high-resolution ultrasonography and are computed as an index of vascular function.[45] During a flow-mediated dilation procedure, the diameter of the brachial artery is measured in response to intentional hyperemia.[48] This increase in sheer stress, assessed after a period of occlusion, induces increased blood flow to the area thereby prompting the release of endothelial-derived nitric oxide. This increase in nitric oxide triggers a vasodilatory response in the target artery.[45,48] It has been demonstrated that factors such as exercise, temperature, drugs, and certain foods can affect flow-mediated dilation measurements. As such, it is recommended that subjects undergoing this procedure fast for at least eight hours prior to examination, and that all assessments be completed in a temperature-controlled environment free of noise. Further, given that vasoactive medications may impact flow-mediated dilation measurements, it is best for subjects to refrain from use for at least four half-lives of the medication.[45] In healthy subjects, flow-mediated dilation values are generally 7-10% of subjects' baseline diameter, however; subjects with cardiovascular diseases tend to have values between 0-5% of their baseline diameter.[49]

Studies which have compared flow-mediated dilation values in healthy individuals versus those with atherosclerosis have found that flow-mediated dilation values were depressed in subjects with atherosclerosis, and other coronary risk factors.[49] Next, flow-mediated dilation values were generally higher in pre-menopausal women and lower in older individuals.[49,53] Specifically, linear regression models conducted on subjects from the Framingham Heart Study indicated that flow-mediated dilation values were positively associated with heart rate and female sex while there was an inverse association specific to age, systolic blood pressure, and BMI.[53] However, it should be noted that, even in healthy

subjects, advanced age appears to have a profound impact on flow-mediated dilation values.[54,55] In fact, a comparison between younger subjects (26.5 +/- 7.2 years old) and elderly subjects (71.3 +/- 5.8 years old), all of whom were reported free of major cardiovascular risk factors, revealed that younger subjects had significantly higher flow-mediated dilation values.[54] Similarly, findings from a recent publication indicated that, for both sexes, flow-mediated dilation values decreased significantly with age. For instance, men experienced a steady decline in flow-mediated dilation after the age of 30 while women experienced the steepest decline after the age of 45.[55]

Considering that flow-mediated dilation measurements are instrumental in capturing cardiovascular responses to both short- and long-term events, it is now regarded as an excellent assessment tool to determine endothelial function following nutrition or physical activity interventions.[49,56] A crossover investigation conducted on 24 relatively healthy subjects to compare the effects of olive oil versus walnut consumption indicated that subjects who ingested walnuts saw a significant increase in flow-mediated dilation values over subjects who ingested olive oil.[56]

Pulse Wave Analysis

In humans, the pulse is an important signal of cardiovascular health.[57] Each pulse originates at the heart and then migrates to the blood vessels.[57,58] As each pulse travels to the blood vessels, reflections may occur at any level within the vessel walls.[57] Several conditions play a role in the rate and/or rhythm of a pulse including the elasticity of blood vessel walls, the viscosity of blood, and the resistance of blood flow.[57] These physiologic changes may influence the reflection of a pulse and/or the strength or frequency of a pulse.[57] While there are numerous techniques used to assess the human pulse, the use of

pulse wave analysis has been longstanding given that these measurements provide valuable information on the cardiovascular system[57] including early detection of arterial stiffness, and the onset and progression of hypertension.[59]

Researchers agree that, independent of blood pressure, arterial stiffness is an important indicator of sub-optimal central-aortic systolic blood pressure, even during early stages of hypertension when symptoms of the disease are not yet present.[44,60,61] With each heartbeat, arteries transport oxygenated blood and other nutrients to the rest of the body including the brain and other vital organs. Given the importance of these transport vessels, it is crucial to understand which factors negatively impact healthy arteries. These factors may include prolonged stress, chronological age,[60] and structural damage to the arteries as a result of unhealthful lifestyle behaviors including tobacco use or other disease states.[44] While healthy arteries are highly elastic and capable of expanding to withstand increased blood flow, unhealthy arteries gradually lose their elasticity, and become stiffer and less pliable over time.[44,60]

A randomized, crossover study conducted on 19 healthy subjects to assess the effects of acute mental stress on arterial stiffness indicated that mental stress was positively associated with sustained increases to central-aortic systolic pressure, pulse pressure, and stiffness of the aortic artery.[62] In another study employing similar methods, laughter was shown to decrease arterial stiffness, while acute stress was shown to have the opposite effect.[63]

Next, a study conducted on almost 3000 subjects indicated that there was an increased risk of heart disease and stroke in the presence of stiffer arteries.[64] Similarly, when researchers assessed the relationship between arterial stiffness and organ damage in

almost 200 subjects with hypertension, arterial stiffness was positively associated with organ damage, as indicated by increased triglycerides, 24-hour pulse pressure, and 24-hour systolic pressure.[65] Finally, a study conducted on over 500 hypertensive subjects who were classified as current-, ex-, or non-smokers revealed that, when compared to non-smoking subjects, current and ex-smokers had a significantly higher level of arterial stiffness.[66]

Pulse Wave Velocity

As previously indicated, hypertension develops as a result of prolonged stiffness within the arterial walls, and researchers agree that arterial stiffness is an important and independent contributor of all-cause cardiovascular risk.[67-70] Currenty, there are several laboratory assessments that may be used to assess the level of stiffness within the arterial walls. However, of these indices, pulse wave velocity of the cartoid-femoral artery is regarded as the gold standard because of its ease of use, reliabilty, and the growing body of evidence that has demonstrated a clear association between pulse wave velocity measurements and cardiovascular disease risk.[67,69-71] It should be noted that, prior to the past five years, utilization of this non-invasive technique has been largely hindered due to a lack of established reference ranges.[67] However, following a landmark study conducted on almost 17,000 subjects with various blood pressure ranges, and/or cardiovascular risk factors such as smoking and dyslipidemia, reference ranges were established based on several different methodologies and equations, and were provided for populations of various age ranges, and health parameters.[67] Unlike flow-mediated dilation parameters, lower pulse wave velocity values suggest less arterial stiffness and increased cardiovascular health. For example, for healthy individuals under the age of 30, optimal pulse wave velocity was recorded at 6.1 m/s (4.6–7.5; 2 SD). Conversely, with increasing age, optimal

pulse wave velocity tended to increase, but the overall goal is to maintain these values as low as possible.[67]

Given these standards, the assessment of pulse wave velocity has been used to better understand how arterial stiffness relates to the development of cardiovascular diseases, and as previously indicated, a higher pulse wave velocity index is associated with a higher level of arterial stiffness and increased cardiovascular risk.[72,73] Findings from a cross-sectional study showed that, among nearly 400 post-menopausal women between the ages of 50 and 74, the presence of arterial stiffness was positively associated with BMI, type 2 diabetes, fasting glucose levels, and triglycerides. As such, the risk of heart disease, stroke, and death were higher among subjects with higher pulse wave velocity scores.[72]

Similarly, advanced age appears to be a major determinant of pulse wave velocity values, and independent of blood pressure level, this occurrence is especially true in women.[73-75] A study conducted on over 200 pre- and post-menopausal women demonstrated that there were significant differences between global pulse wave velocity when comparing pre- and post-menopausal women. More specifically, post-menopausal women had higher global pulse wave velocity values when compared to pre-menopausal women. Next, similar differences were observed in pre-menopausal women who were normotensive versus those who were hypertensive, where pre-menopausal women who were normotensive had lower global pulse wave velocity values when compared to pre-menopausal women who were hypertensive.[75]

In another study conducted on post-menopausal women, investigators utilized a pulsed doppler global pulse wave velocity technique to assess the relationship between aortic stiffness and cardiovascular risk in nearly 300 subjects. Here, subjects were

categorized by diabetes, hypertension, hypercholesterolemia, or any combination of these conditions.[74] Findings from this trial revealed that a combination of all three conditions was the significant determinant for a higher global pulse wave velocity index over single diagnoses. These findings further support the association between aortic stiffness and cardiovascular risk factors in post-menopausal women.[74]

Nitric Oxide

Nitric oxide, synthesized by endothelial cells from the precursor L-arginine, is a signaling molecule that plays an important role in regulating the cardiovascular system as well as the nervous and immune systems.[76,77] Specific to the cardiovascular system, nitric oxide has long been recognized as a key regulatory molecule that influences resting vascular tone, vasodilation of arterial walls, platelet adhesion, regeneration of endothelial tissue, and inhibition of white blood cells, when necessary.[77,78]

Generally speaking, individuals with hypertension exhibit marked increases in vascular tone and reduced vasodilation of large, conduit arteries.[23,78] This continued form of vessel damage may result in vascular remodeling that limits the bioavailability and bioactivity of nitric oxide.[78] A previous investigation indicated that nitric oxide synthesis is diminished even in early stages of primary hypertension, and that diminished nitric oxide synthesis is also detectable in young, normotensive offspring of subjects with hypertension.[79] However, it should be noted that there is no direct evidence to suggest that offspring of hypertensive subjects experience higher blood pressure as a result of diminished nitric oxide synthesis.[79]

Additionally, it is also important to recognize that nitric oxide-promoting anions such as organic nitrates and nitrites may be diminished in hypertensive environments and

that smooth muscles may become insensitive to circulating nitric oxide.[80,81] A study conducted to examine the relationship between various stages of essential hypertension and plasma nitrate and nitrite concentrations reported that, when compared to subjects in the control group, subjects with hypertension have significantly lower levels of plasma nitrates, nitrites, and nitric oxide. Further, there was a significant, inverse relationship between nitric oxide concentrations and systolic and diastolic blood pressure levels.[23]

Regulation of the Nitric Oxide Pathway

Historically, nitrates and nitrites were regarded as inactive derivatives of nitric oxide metabolism with potentially cancer-causing effects.[17] However, investigations conducted during the past ten years oppose this position, and argue that these inorganic anions are, in fact, beneficial to the human body.[17] More specifically, studies have shown that nitrates and nitrites may be recycled in the blood and tissues to upregulate nitric oxide synthesis in oxygen-deficient situations.[17,82,83] Given the ability of these anions to stimulate nitric oxide bioactivity, it is essential to further investigate the mechanisms by which these anions protect against nitric oxide-regulated health conditions such as hypertension.[17]

Following the reduction of dietary nitrates to nitrites, various pathways, including the L-arginine-NOS pathway and the nitrate-nitrite-nitric oxide pathway may be present to further reduce nitrites to nitric oxide. However, during instances of reduced oxygen availability, a condition known as hypoxia, the L-arginine-NOS pathway is down-regulated while the nitritate-nitrite-nitric oxide pathway becomes the primary means by which nitric oxide is produced. Thus, in instances of hypoxia, the production of nitric oxide is significantly enhanced to aid in vasodilation, and the regulation of cellular respiration.[17,84,85]

Bacteria Needed for the Nitric Oxide Pathway

To initiate the process of nitric oxide production via the nitrate-nitrite-nitric oxide pathway, dietary nitrate must first undergo an initial reduction to nitrite via commensal bacteria present in the mouth. Facultative anaerobic bacteria is necessary because humans lack the necessary reductase enzymes to reduce nitrate to nitrite.[17,86] Following consumption, dietary nitrate undergoes rapid absorption in the upper gastrointestinal tract. From here, the salivary glands extract up to 25% of circulating nitrate which is then concentrated in the saliva. Next, facultative anaerobic bacteria in the oral cavity reduces nitrate to nitrite via the action of nitrate reductase enzymes present in the bacteria. Once the nitrite-rich saliva is swallowed and reaches the stomach, the nitrite present in the system is converted to nitrous acid which further decomposes to form nitric oxide.[86-88]

Role of Nitrates in Hypertension

Nitrates are an important form of naturally occuring nitrogen that is of particular importance to the nitrogen cycle.[87] In healthy individuals, normal plasma levels of nitrates range from 20-40 μM/L while nitrite concentrations are significantly less and range from 50-300 nM/L.[17,88] Further, plasma levels of both nitrates and nitrites increase substantially in the presence of inflammatory diseases.[17] However, in instances of endothelial dysfunction, such as the case in hypertension, levels of both anions remain low.[88] For instance, when compared to baseline levels of nitrites, there appears to be a progressive and significant decline in nitrite levels as cardiovascular risk increases. Similarly, when compared to healthy individuals, those with endothelial dysfunction experience lower levels of flow-mediated dilation and plasma nitrite as these two factors are positively and significantly correlated.[88] Next, emerging evidence indicate that foods high in inorganic

nitrates may be beneficial for various measurements of cardiovascular function including the ability to reduce blood pressure, improve endothelial function, and reduce dislipidemia.[71] Thus, increasing the consumption of high-nitrate foods may very well prevent or reduce the likelihood of developing other cardiovascular diseases.[15,71,89] More importantly, increased consumption of high-nitrate vegetables and low-fat dairy products may be effective for lowering blood pressure to a similar extent as single-class hypertension medications.[92]

How Nitrates Protect Against Hypertension

As previously indicated, nitrates were regarded as undesirable byproducts of nitric oxide metabolism.[17,93] However, recent data have demonstrated the importance of dietary inorganic nitrates to guard against hypertension in several study populations. The mechanism by which nitrates may prevent hypertension is via the upregulation of nitric oxide synthesis. An increase in nitric oxide synthesis is important to allow the arteries to dilate during periods of sheer stress resulting in decreased blood pressure, and improved overall cardiovascular function.[17,91,93] In order for dietary nitrates to be used as a part of the nitric oxide pathway to promote vasodilation of the arterial walls, this important anion must first be reduced to nitrite within the body.

Given that humans lack the specific reductase enzymes to effectively convert nitrates to nitrites, a commensal facultative anaerobic bacteria located in the upper gastrointestinal tract and in the oral cavity is required to successfully reduce nitrate to nitrite.[17,93] Once this conversion occurs, nitrites are eventually reduced to nitric oxide via several mechanisms. Additionally, in instances of hypoxia and increased acidosis, the

synthesis of nitric oxide is greatly enhanced to ensure that the body maintains normal functioning.[17,93]

To date, numerous trials have indicated that upregulating nitric oxide, either through endogenous means or via the administration of nitric oxide precursors, is effective to increase nitric oxide production and subsequently decrease blood pressure.[78,94-96] In one trial, exogenous L-arginine, a precursor of nitric oxide, was administered intravenously to subjects with primary and secondary hypertension. Following administration, subjects' blood pressure, heart rate, cardiac output, and several other functional markers were assessed. Findings from this investigation showed that nitric oxide increased following administration of L-arginine, and mean arterial blood pressure and peripheral resistance decreased while cardiac output and heart rate increased.[94] Next, a crossover trial assessing the intake of supplementary beetroot juice administered to healthy young subjects demonstrated a significant reduction of approximately 4% in both systolic and diastolic blood pressure, which was maintained for roughly two weeks with continued administration of the intervention.[95] In other studies, ingestion of high-nitrate food or beverage items resulted in increased bioactivation of nitric oxide which led to vasoprotective outcomes in as little as three hours after ingestion.[95,97] Further, ingestion of a high-nitrate meal has been shown effective for preventing acute endothelial dysfunction, measured by purposefully inducing an ischemic assault to the forearm.[97]

Nitrate-Rich Foods

Dietary constituents that are notably high in nitrates include green, leafy vegetables such as spinach, arugula, celery, and lettuce; root vegetables such as red beetroot, turnip, and radish; and herbs such as parsley, and fennel.[71,91] In addition to vegetables and select

fruits, nitrates are naturally-occurring in the human body, and may even be formed via a combination of fertilizers, manure, and plant remains.[89,91] As such, high concentrations of nitrates may be present in the air, soil, plant fertilizer, and water. Additionally, nitrates have been historically used as a preservative to prevent microbial growth and spoilage, and are generally found in raw and processed meats including hot dogs, bacon, select cheeses, and some alcoholic beverages.[89]

In spite of the origin of nitrates, there is considerable variability in nitrate content among foods.[91] Specific to vegetables, much of this variability is attributable to light exposure and the amount of nitrogen present in the fertilizer used before or during harvest.[98,99] For instance, a convenience study which assessed the nitrate content of various fruits and vegetables found that spinach showed the highest concentration of nitrates followed by mustard greens. Conversely, fruits including bananas and oranges exhibited the lowest concentation of nitrates. Aside from the sample of fruits, vegetables, and select cured meats assessed in the study, a commercially-prepared vegetable supplement showed the highest concentration of nitrates.[91]

Studies that have explored the effects of high-nitrate foods on cardiovascular function have shown promise in various study populations.[71,91] For instance, a 3-day randomized, placebo controlled crossover study which assessed the effects of a high nitrate diet supplement on blood pressure in healthy, physically active young adults demonstrated decreased levels of diastolic blood pressure following ingestion of the nitrate supplement.[12] Further, findings from this study were similar to that of the well-known DASH trial.[100] In another randomized crossover study, which sought to assess the short-term effects of green leafy vegetables on blood pressure and arterial stiffness in a sample of subjects with high-

normal blood pressure (120 to 139 mm Hg), subjects who consumed the high-nitrate intervention for seven days had significantly increased salivary and plasma nitrate/nitrite concentration when compared to subjects who consumed the low-nitrate intervention. However, no significant findings were reported for ambulatory or home blood pressure measurements or for indices of arterial stiffness in this sample population.[71]

Bioavailability of Nitrates

Roughly 80-85% of all nitrates consumed are in the form of vegetables.[101] Further, following ingestion, the absorption rate of dietary nitrates is relatively quick and approximately 20-28% of the ingested load is secreted into the saliva.[101,102] Additionally, several sources report that the bioavailability of nitrates is approximately 100% regardless of the form consumed (e.g., cooked versus uncooked varieties).[91,101] A recent crossover trial, which assessed plasma levels of nitrates following ingestion of cooked spinach, raw lettuce and cooked beetroot, indicated that the bioavailability of nitrates was similar across the three treatment groups. That is, the absolute bioavailibility of nitrates was 98 +/- 12% and 106 +/- 15% from cooked spinach and cooked beetroot, respectively, while the absolute bioavailability of nitrates from raw lettuce was 114 +/- 14%.[101]

Nitrate Contraindicators

In spite of the high biovailability of nitrates,[101] there are several agents that limit or altogether inhibit the ability for nitrates to be converted to nitrite, and eventually to the vasodilator, nitric oxide.[103-105] One such contraindicator is strong mouthwash solutions. While the use of mouthwash agents have been recommended to reduce and/or to treat halitosis and to enhance oral health, the use of strong antibacterial mouthwash solutions

may be potentially harmful to cardiovascular health due to the fact that these solutions limit the bioavailability of nitric oxide by impairing the nitrate-nitrite-nitric oxide pathway.[103]

Recently, a randomized study, which sought to examine the effects of three different strengths of mouthwash solutions on salivary and plasma nitrate concentrations in healthy, normotensive individuals indicated that the administration of Listerine mouthwash (an antiseptic agent) had no discernable effect on the conversion of nitrates to nitrites. This is perhaps due to the fact that the solution contained a relatively low amount of antibacterial agents to actually impact the bacteria present in the mouth and saliva. Conversely, there appeared to be a greater effect of the antibacterial mouthwash solution, and chlorhexidine mouth rinse. Specifically, the antibacterial agent appeared to limit the conversion of plasma nitrates to nitrites, while the chlorhexidine rinse altogether inhibited the conversion of plasma nitrates to nitrites.[103]

Other trials utilizing similar study designs have reported comparable results.[104,105] For instance, a study assessing the effects of weak versus strong antibacterial mouthwash agents reported that, following ingestion of a nitrate treatment in healthy adults, both agents were effective for reducing the rise of plasma nitrites. Further, use of the strong mouthwash solution appeared to increase mean systolic blood pressure during a low-intensity treadmill test conducted four hours following ingestion of the nitrate load.[104] Another study conducted on older subjects with hypertension (mean age of 65) indicated that relative to the control group, subjects who used the antibacterial mouthwash exhibited a decreased ability to convert nitrates to nitrites, and showed higher systolic blood pressure levels following the 3-day intervention period.[105]

Another factor that has been shown to impact nitric oxide regulation is ethanol, an active ingredient present in beverages including beers, wines, and strong liquors. Early animal studies revealed that nitric oxide synthesis may be limited by the consumption of ethanol.[106] However, in human trials, the effects of ethanol on the endothelium may be somewhat more complex. Specifically, low-dose ethanol consumption appears to have favorable effects on endothelial function by upregulating nitric oxide production. Conversely, high-dose ethanol exposure appears to have a negative effect on endothelial function by reducing nitric oxide production.[107,108]

Lifestyle Factors Affecting Blood Pressure

A report published by the *Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure* declared that the risk of developing cardiovascular diseases begins at a systolic blood pressure of 115 mm Hg and a diastolic blood pressure of 75 mm Hg. Further, risk appears to increase twofold with each 20/10 mm Hg increase in blood pressure.[109] The report further indicated that normotensive individuals, aged 55 years old, have a 90% risk of developing hypertension over their lifetime, and that efforts should be made to promote lifestyle initiatives to treat and prevent the development of this chronic condition.[109] Additionally, maintaining a 12 mm Hg decrease in systolic blood pressure for a period of ten years may be effective for preventing one out of eleven deaths in patients treated with hypertension.[109] To date, notable factors that have been shown to influence blood pressure are exercise, anti-inflammatory agents, and diet.[109-114]

The Effects of Exercise on Blood Pressure

Prolonged inactivity has been positively associated with increased risk of hypertension and other chronic conditions including obesity, type 2 diabetes, as well as joint and bone diseases,[113,114] and these findings have been consistent among various study populations. Thus, physical inactivity has long been regarded as a modifiable risk factor for these chronic conditions,[110,114] and studies which have assessed the effects of physical activity on the risk of death from cardiovascular diseases have noted that continuous involvement in physical activity is associated with greater than 50% risk reduction.[112] Further, individuals who increased their energy expenditure (of up to 1000 kcal per week) through physical activity involvement showed a 20% reduction in mortality risk.[111] Conversely, middle-aged women who reported high levels of sedentary behavior (defined as less than one hour of physical activity per week) exhibited a twofold increase in risk for cardiovascular diseases, including hypertension, and a 52% increase in all-cause mortality risk.[111]

Considering that low levels of cardiorespiratory fitness are associated with both all-cause mortality risk and risk for cardiovascular diseases, researchers have taken a keen interest in understanding the role that physical activity plays in improving cardiovascular fitness and reducing overall mortality risk.[114-117] In a randomized, controlled trial of roughly 150 overweight or obese subjects with pre-hypertension or stage I hypertension, regular involvement in physical activity resulted in significant reductions in both clinical and ambulatory blood pressure (approximately 16.1/9.9 mm Hg reduction). Furthermore, subjects developed significant improvements in pulse wave velocity and other cardivascular function measures.[115] Meta-analyses conducted on both aerobic exercise and

resistance training routines indicate that both forms of physical activity have been shown to be effective for reducing blood pressure in various study populations. However, of the two forms of activity, there is considerably more evidence to support the role of aerobic exercise in blood pressure reduction.[116,117]

The Effects of Anti-inflammatory Agents on Blood Pressure

In an effort to relieve pain and discomfort associated with acute and chronic illnesses, the use of anti-inflammatory agents have become a common treatment option. However, a growing body of evidence suggests that certain anti-inflammatory agents may actually promote the onset of diseases such as hypertension, myocardial infarction, heart failure, and stroke.[118,119] For example, in 2004, the drug Rofecoxib (Vioxx), generally prescribed to relieve pain associated with osteoarthritis, was shown to increase the risk of cardiovascular events, and was subsequently withdrawn from wordwide use. Later that same year, the US Food and Drug Administration (FDA) issued a warning for valdecoxib (Bextra), a drug used to relieve painful menstruation cramps and various forms of arthritis, given that use of this drug was contraindicated in individuals undergoing coronary bypass graft surgery. Similarly, the National Institutes of Health (NIH) suspended the use of celecoxib (Celebrex), a drug that inhibits inflammatory hormones that are present during menstruation and arthritis, as it was also linked to increased risk of cardiovascular events.[119]

Additionally, oral anti-inflammatory agents such as pseudoephedrine are commonly used to treat symptoms associated with rhinitis and rhinorrhea. To date, many over-the-counter and prescription decongestants include pseudoephedrine. Examples include Allegra-D, AlkaSeltzer Plus Cold Medicine, Aleve Cold and Sinus Caplets, Claritin-D 24-

hour Tablets, Benadryl Allery and Sinus Tablets, and Sudafed 24-hour Tablets.[120] A review article published in 2005 aimed at understanding the effects of pseudoephedrine use on blood pressure and heart rate yielded interesting results.[120] First, there appeared to be a small, but significant increase in systolic blood pressure and heart rate following the use of agents containing pseudoephedrine. Next, immediate-release of pseudoephedrine appeared to significantly elevate systolic blood pressure while sustained-release of the agent showed no significant effect. Conversely, heart rate appeared to increase significantly following both immediate-release and extended-release formulations. Finally, higher doses of pseudoephedrine were linked to substantial elevations of both systolic and diastolic blood pressure and subjects with stable, treated hypertension experienced increased systolic blood pressure, but no effect on heart rate and diastolic blood pressure as a result of pseudoephedrine therapy.[120]

Given the link between the use of anti-inflammatory agents and the risk for cardiovascular events, the American Heart Association (AHA) issued a statement to guide clinicians on how to best balance the risks and benefits associated with the use of these agents. AHA's statement indicates that individiuals with prior history of cardiovascular diseases, or those with prior risk for these diseases are at greatest risk for adverse effects of anti-inflammatory agents. Further, for these at-risk individuals, anti-inflammatory agents should only be prescribed in the event that no alternative medications exist. Finally, the AHA recommmends that the lowest dose of anti-inflammatory medications be prescribed for the least amount of time.[119]

Dietary Factors Affecting Blood Pressure

A growing body of evidence has focused on the relationship between diet and blood pressure, and given that progressive elevations in blood pressure are associated with the onset of hypertension,[7] it is becoming clear that efforts should be taken to fully grasp the complex relationship between dietary behaviors and blood pressure. To date, sub-optimal diet quality has been highlighted as the leading risk factor for disability and death in the U.S. and in 2010, 678,000 deaths were linked to poor diet quality including insufficient fruit, vegetable, and whole grain intake as well as excess consumption of sodium.[1] Given these statistics, numerous researchers have focused their attention on identifying food items that may be beneficial for reducing elevated blood pressure[121] while other researchers have directed their attention on foods that may be adversely associated with blood pressure. In such studies, several themes have emerged including the relationship between potassium and blood pressure, sodium and blood pressure, caffeine and blood pressure, and vitamin C and blood pressure.

The Effect of Potassium on Blood Pressure

In humans, potassium is the most abundant cation. It is primarly contained in the cells but a small percentage can be found in extracellular fluid.[122] In healthy individuals, approximately 90% of potassium is absorbed and then excreted by the kidneys. However, multiple factors, such as the activation of potassium channels, influence the secretion of potassium.[122,123] Once potassium channels are activated, endothelial cells and vascular tone may be regulated. Findings from animal studies have demonstrated that supplementation of potassium is effective for improving endothelial function by increasing the production of nitric oxide.[124] Additionally, supplementation of potassium may cause a stimulation of

potassium channels resulting in vasodilation of arteries.[125] In human subjects, a meta-analysis of more than thirty randomized controlled studies have also reported the importance of potassium consumption for blood pressure regulation. More specifically, low potassium intake may lead to increased levels of blood pressure while increased levels of potassium may prevent or treat sub-optimal blood pressure levels.[121,126]

The Effect of Sodium on Blood Pressure

A mineral of considerable interest in blood pressure regulation is sodium. In the U.S. alone, approximately 58,000 annual deaths are linked to excess sodium consumption (greater than 2.0 grams per day). This accounts for 1 in 16 overall deaths from cardiovsacular disease and 1 in 8 deaths from cardiovascular disease in individuals 70 years old or younger. Globally, nearly 1 in 10 individuals or 1.65 million annual deaths were attributable to excess sodium consumption.[1] While there is no denying that excess sodium is deleterious to health, adequate amounts of this mineral are important to maintain homeostasis. Specifically, sodium plays an essential role in maintaining pH balance, regulating extracellular fluid, and is a key cation involved in controlling cellular function across multiple organ systems. Additionally, sodium is important to ensure arterial pressure and adequate blood volume.[127,128] Under normal physiological conditions, decreasing sodium intake is crucial for decreasing blood volume, inducing the release of renin, and subsequently producing angiotensin II. Taken together, renin and angiotensin II hormones result in a vasoactive response thereby constricting blood vessels, when necessary.[126]

Findings from a longitudinal population study conducted on almost 1500 participants with high blood pressure over a six-year period showed that, on average, there were significant annual increases in blood pressure.[129] Specifically, systolic blood pressure

increased by approximately 0.37 mm Hg while diastolic blood pressure increased by roughly 0.47 mm Hg. In spite of increases in overall blood pressure, there were no significant changes in 24-hour urinary excretion of sodium over the course of the trial. Next, a randomized, controlled cross-sectional study conducted to assess the effects of sodium restriction on blood pressure in subjects with resistant hypertension indicated that excessive dietary sodium contributed to resistance to anti-hypertensive medication.[129] Next, a landmark multi-center cross-sectional study conducted to examine the effects of sodium consumption on blood pressure indicated that there was a positive association between high levels of sodium intake and blood pressure. Specifically, increased dietary sodium led to significantly higher systolic and diastolic blood pressures.[130]

The Effect of Caffeine on Blood Pressure

In addition to potassium and sodium, another agent that has been linked to blood pressure regulation is caffeine. While the majority of previous studies which have assessed the effects of caffeine consumption on blood pressure have been primarly conducted on healthy populations, it is important to note that there are several landmark investigations conducted on hypertensive populations.[131,132] For example, a randomized crossover trial showed that consumption of caffeine resulted in significant increases in both systolic and diastolic blood pressures in post-menopausal women undergoing hormone replacement therapy.[133]

Next, a systematic review conducted on hypertensive subjects indicated that increased caffeine consumption of 200-300 mg resulted in a mean increase of systolic blood pressure by 8.1 mm Hg and by 5.7 mm Hg for diastolic blood pressure. Further, increases in blood pressure were observed during the first hour of intake, and remained

elevated for ≥3 hours. However, there were no significant differences in blood pressure seen in studies assessing the longer-term effects (approximately two weeks) of coffee, decaffeinated coffee, and caffeine-free beverage consumption.[134]

The Effect of Vitamin C on Blood Pressure

Ascorbic acid, more commonly known as vitamin C, is a micronutrient abundant in many fruits, vegetables, dietary supplements, fortified ready-to-eat cereals, and select beverages.[135] To date, researchers agree that vitamin C is effective for reducing oxidative stress and for enhancing endothelial function via the production of nitric oxide. In fact, as early as the mid-1940s, vitamin C supplementation was thought to result in anti-hypertensive effects in human trials.[135,136] Further, it has also been suggested that increased levels of vitamin C may reduce blood pressure.[137]

A systematic review of randomized controlled trials conducted to determine the relationship between vitamin C supplementation and blood pressure found significant reductions in both systolic and diastolic pressures. An 8-week randomized, double-blind, placebo controlled crossover trial indicated that hypertensive subjects had a reduction of systolic blood pressure by approximately 24.85 mm Hg and a reduction of diastolic blood pressure by approximately 24.85 mm Hg following vitamin C supplementation.[135,138] Next, long-term supplementation (for a duration of approximately three months) of vitamin C appeared to affect blood pressure somewhat similarly. Specifically, a double-blind, randomized placebo-controlled crossover trial conducted on older men and women (aged 60 and above) showed a modest, but significant decrease in ambulatory systolic blood pressure.[139] Similarly, in subjects with essential hypertension, vitamin C supplementation appeared to be an effective method for improving impaired endothelial

by reversing the effects of free radicals which are associated with endothelial dysfunction.[79]

Summary

Overall, a significant amount of research has been conducted to better understand the complex relationship between food and the prevention of hypertension and hypertension-related ailments in various study populations. Despite this forward progress, many aspects of this chronic condition remain unknown especially as it relates to the consumption of nitrate-rich dietary sources. Further, the overall prevalence of hypertension continues to increase, a trend that is primarily driven by post-menopausal women.[140] Thus, given the apparent gaps in the current body of literature pertaining to the effects of nitrate-rich dietary sources and cardiovascular health in post-menopausal women, our randomized, placebo-controlled, crossover trial sought to compare the effects of a fresh nitrate-rich vegetable salad to a canned low-nitrate vegetable medley, consumed twice per day, on plasma nitrate/nitrite concentration, peripheral and central-aortic systolic and diastolic blood pressures, pulse wave velocity, and flow-mediated dilation in healthy, post-menopausal women residing in Phoenix, Arizona.

CHAPTER 3

METHODS

Study Design

A randomized, placebo-controlled, crossover trial was conducted at Arizona State University (ASU) from June 2016 to August 2016. This feeding trial compared the effects of fresh nitrate-rich vegetable salads versus canned low-nitrate canned vegetables on plasma nitrate/nitrite concentration, peripheral and central-aortic systolic and diastolic blood pressures, pulse wave velocity, and flow-mediated dilation in healthy, post-menopausal women residing in Phoenix, Arizona. Approval for this project was granted by the Institutional Review Board (IRB) at Arizona State University on May 23, 2016 (Appendix A).

Participants

Subject Selection

Healthy, post-menopausal women were recruited from Arizona State University (ASU) campuses and surrounding cities in the Phoenix Metropolitan area. Written informed consent was obtained from all subjects prior to enrollment into the trial (Appendix B).

Inclusion and Exclusion Criteria

Inclusion criteria for this trial were healthy, post-menopausal women regardless of race/ethnicity, weight status, BMI classification, and socioeconomic status with blood pressure levels ($\geq$ 115/70 mm Hg and < 140/80 mm Hg), subjects not taking hypertensive medication, non-smokers, subjects not consuming alcoholic beverages, multi-vitamins,

antihistamines, decongestants, and those not using antiseptic/antibacterial mouthwash for the duration of the trial. Subjects who failed to meet these parameters were excluded from the trial.

Recruitment Strategies

Subjects were recruited from the ASU Phoenix, West, Tempe, and Polytechnic campuses via online and in-person flyers in June and July of 2016. Recruitment efforts were extended to public settings including libraries within surrounding cities as well as various online platforms. Flyers informed potential subjects that the 8-week trial was designed to examine whether blood vessel function and blood pressure would improve following the consumption of a vegetable salad versus a vegetable medley consumed twice per day during the treatment phases (Appendix C). An invitation to complete an online pre-screening questionnaire was extended to interested subjects (Appendix D), and qualifying individuals were invited to meet with study personnel at the ASU Downtown Phoenix campus to further discuss the trial.

Study Protocols

A randomized, placebo-controlled, crossover trial was employed to assess study outcomes (Appendix E). Pre-screening occurred via an online questionnaire and prior to the beginning of the trial subjects visited the Arizona Biomedical Collaborative (ABC) building in Phoenix. During this visit (visit one), further screening (e.g., Health History Questionnaire (HHQ) (Appendix F), Food Frequency Questionnaire (FFQ) (Appendix G) and blood pressure) was completed, and eligible subjects were asked to refrain from antiseptic/antibacterial mouthwash for the duration of the study. Additionally, subjects were asked to refrain from multi-vitamins, antihistamines, and decongestants three days

prior to study visits two through five and from alcoholic beverages, caffeine, and heavy exercise 12-hours prior to study visits two through five. Next, instructions were given to subjects to maintain their usual diet and exercise routine, and to restrict medium- and high-nitrate fruits and vegetables (list provided) for the duration of the study. Further, subjects were randomized to either the intervention (high-nitrate: ~445 mg nitrates per day) or the placebo (low-nitrate: <50 mg nitrates per day) group, and a study protocol checklist was maintained by the investigators (Appendix H). The vegetable medley consisted of canned, low-nitrate vegetables with little- or no-added salt. Given the crossover design of the trial, the following protocols were followed for all subjects during visits two through five.

At visit 2, baseline measurement of blood pressure, pulse wave analysis, pulse wave velocity, flow-mediated dilation, and plasma nitrates were obtained. At visit 3, scheduled at the end of the 10-day feeding period, measurements obtained at visit 2 were repeated. Following the washout period (two to three weeks in duration), visit 4 was scheduled. At visit 4, peripheral blood pressure, central aortic blood pressure, pulse wave velocity, flow-mediated dilation, and plasma nitrate/nitrite measurements were obtained, and subjects were given either a 10-day supply of the high-nitrate vegetable salad or a 10-day supply of the vegetable medley. Finally, visit 5 occurred following the second 10-day feeding period, and peripheral blood pressure, central aortic blood pressure, pulse wave velocity, flow-mediated dilation, and plasma nitrate/nitrite measurements were once again obtained. In totality, measurements of central aortic blood pressure, pulse wave velocity, flow-mediated dilation, and plasma nitrates/nitrites were obtained at four time points: prior to the first trial phase, and following each trial phase. Conversely, peripheral

blood pressure and weight were collected at five time points: during the screening visit, and prior to and after each of the two feeding periods.

Outcome Measures

Plasma Nitrate/Nitrate Concentration

Subjects provided a fasting blood sample at each of the four time points. Samples were collected into EDTA vacutainers, and plasma was separated by centrifugation. Plasma samples were then filtered using 30 kDa MWCO micro-centrifuge filters to remove hemoglobin and large plasma proteins. Nitrate/nitrite concentration in plasma samples were then measured using a commercially available kit (Cat. No. 780001, Cayman Company, Ann Arbor, MI) according to the instructions.

Peripheral Blood Pressure

Upon entering the study laboratory, subjects were asked to lie down in a dimly lit room where their resting peripheral blood pressure (systolic and diastolic) was measured at zero and ten minutes to ensure hemodynamic stability using an Omron® Auto Cuff Blood Pressure Monitor. The Omron® one-touch blood pressure monitor was equipped with IntelliSense technology developed by Omron Healthcare to inflate and deflate at the optimum level depending on the individual user's arm size. Next, a non-invasive SphygmoCor XCEL (AtCor Medical, Sydney, NSW, Australia) system was employed to measure peripheral blood pressure. Subjects' peripheral blood pressure was obtained a total of three times and the average of the last two measurements was recorded to determine the final peripheral blood pressure.

Central-aortic Blood Pressure

Following peripheral blood pressure measurements, a non-invasive SphygmoCor XCEL (AtCor Medical, Sydney, NSW, Australia) system was used to measure pulse wave analysis to derive a central aortic systolic and diastolic blood pressure reading. A blood pressure cuff attached to the SphygmoCor XCEL system was placed around subjects' non-dominant upper arm above the elbow. The SphygmoCor XCEL device first obtained subjects' resting heart rate and resting blood pressure; next the system captured subjects' brachial waveform. By utilizing the brachial generalized transfer function of the device, data on the (1) central-aortic waveform, (2) central-aortic systolic pressure, (3) central-aortic pulse pressure, and (4) an augmentation index were obtained. Subjects' pulse wave analysis was obtained a total of three times and the average of the last two measurements was recorded to determine the final central-aortic blood pressure.

Pulse Wave Velocity

Following pulse wave analysis measurements, the non-invasive SphygmoCor XCEL (AtCor Medical, Sydney, NSW, Australia) system was employed to determine carotid-femoral pulse wave velocity, a measure of arterial stiffness. During this procedure, a blood pressure cuff was placed around each subject's upper thigh and carotid and femoral pulse rate was identified. Next, distances from the femoral pulse to the topmost point above of the blood pressure cuff; from the sternal notch to the topmost point above the blood pressure cuff; and from the carotid pulse to the sternal notch were measured and entered into the SphygmoCor XCEL software. Afterward, a pen-like tonometer was placed directly above the carotid pulse in the neck, and firm and stable pressure was applied until the SphygmoCor XCEL device computed carotid-femoral

arterial stiffness. To assess arterial stiffness, the SphygmoCor XCEL device determines the time it takes for the pulse to travel from the carotid artery in the neck to the femoral artery in the thigh. Subjects' pulse wave velocity measurements were obtained a total of three times and the average of the last two measurements was recorded to determine carotid-femoral arterial stiffness.

Flow-mediated Dilation

Flow-mediated dilation of the brachial artery was obtained via a resolution 2D and Doppler ultrasound (Terason uSmart3300 ultrasound, Terason Ultrasound, Burlington, MA) with a linear-array transducer at a transmit frequency of 15 MHz. Following a fifteen-minute resting period, images of each subject's brachial artery were captured while lying on their back in a comfortable position with the non-dominant arm extended and immobilized. Images were captured from a longitudinal plane near the antecubital fold, and were analyzed and recorded.

Statistical Analyses

SPSS (Statistical Package for the Social Sciences) v. 23 was utilized for all statistical analyses. Findings were considered significant at a p-value < 0.05. Results were expressed as mean ± standard deviation. Due to the small sample size (n=5), the researchers were unable to determine if the normality assumption was met. Thus, non-parametric Wilcoxon Signed-Rank tests were utilized to compare mean differences between conditions. Additionally, effect sizes were included for all outcome measures.

CHAPTER 4

RESULTS

Subject Characteristics and Demographics

Of the interested subjects (n=25), (n=17) subjects met the inclusion criteria and were invited to meet with study personnel for the initial screening visit. Of the eligible subjects (n=9), a total of five subjects completed the pilot study and communicated that they consumed the vegetable salad or canned vegetable medley during the assigned feeding phases of the trial. Consumption of vegetable salads and canned vegetable medley was confirmed by compliance calendars. Descriptive statistics, as illustrated in Table 1, indicate that the mean age of subjects at screening was 52.6 ± 5.7 years. As expected, there were no changes in age during the trial. The mean weight at screening was 68.5 ± 17.7 kg, and there were no significant fluctuations in weight following the high- or low-nitrate phases of the study (p=0.757 and p=0.959, respectively).

Table 1. Subject characteristics and demographics

	Minimum	**Maximum**	**Mean**
Age (years)	44.0	60.0	52.6 ± 5.7
Height (cm)	160.0	168.0	163.4 ± 2.9
Weight (kg)	52.1	94.9	68.5 ± 17.7
Body fat (%)	20.9	48.5	33.7 ± 11.2
PA score	15.0	102.0	60.8 ± 33.5

These data were based on n=5 subjects. Descriptive statistics were performed using SPSS Statistical Analysis system 23.0. Data are expressed as mean ± standard deviation. Data are considered statistically significant at p-value < 0.05, and p-values were provided for change in weight following the low and high nitrate phases of the trial.

Plasma Nitrate/Nitrite Concentration

It was hypothesized that subjects would have a higher plasma nitrate/nitrite concentration after consuming a fresh high-nitrate vegetable salad twice per day than

after consuming a canned vegetable medley twice per day. As outlined in table 2, subjects had a higher plasma nitrate/nitrite concentration after consuming a fresh high-nitrate vegetable salad twice per day (*pre-intervention* = 40.8 ± 15.3 µM/L; *post-intervention* = 100.0 ± 40.6 µM/L) than after consuming a canned vegetable medley twice per day (*pre-control* = 51.9 ± 18.8 µM/L; *post-control* = 46.7 ± 17.8 µM/L). When comparing the effects of the high-nitrate and the low-nitrate conditions, there was a statistically significant increase in plasma nitrate/nitrite concentration ($p = 0.043$) following twice daily consumption of the fresh, nitrate-rich vegetable salad. Given these preliminary findings, the hypothesis was supported.

Table 2. A comparison of plasma nitrate/nitrite concentration between the high and low nitrate phases of the study

	High Nitrate	Low Nitrate	P-value	Effect Size
Plasma Nitrate/Nitrite (µM/L)				
Pre	40.8 ± 15.3	51.9 ± 18.8		
Post	100.0 ± 40.6	46.7 ± 17.8		
Δ	59.5 ± 33.4	-5.2 ± 18.0	0.043*	0.640

These data were based on n=5 subjects. Statistics were performed using SPSS Statistical Analysis system 23.0. All values represent mean ± Standard Deviation (SD). Data are considered statistically significant at p-value < 0.05.

Peripheral Systolic Blood Pressure

It was hypothesized that subjects would have lower peripheral systolic blood pressure after consuming a fresh high-nitrate vegetable salad twice per day than after consuming a canned vegetable medley twice per day. As outlined in table 3, subjects had lower peripheral systolic blood pressure after consuming a fresh high-nitrate vegetable salad twice per day (*pre-intervention* = 118.7 ± 9.2 mm Hg; *post-intervention* = 116.6 ± 9.8 mm Hg) than after consuming a canned vegetable medley twice per day (*pre-control* = 123.3 ± 6.8 mm Hg; *post-control* = 127.7 ± 11.6 mm Hg). When comparing the change

in peripheral systolic blood pressure in the high-nitrate and the low-nitrate conditions, there were no statistically significant differences in peripheral systolic blood pressure (p = 0.345). Given these preliminary findings, the hypothesis was not supported.

Table 3. A comparison of peripheral systolic blood pressure between the high and low nitrate phases of the study

	High Nitrate	Low Nitrate	P-value	Effect Size
Peripheral SBP (mm Hg)				
Pre	118.7 ± 9.2	123.3 ± 6.8		
Post	116.6 ± 9.8	127.7 ± 11.6		
Δ	-2.1 ± 6.2	4.4 ± 8.0	0.345	0.299

These data were based on n=5 subjects. Statistics were performed using SPSS Statistical Analysis system 23.0. All values represent mean ± Standard Deviation (SD). Data are considered statistically significant at p-value < 0.05.

Peripheral Diastolic Blood Pressure

It was hypothesized that subjects would have lower peripheral diastolic blood pressure after consuming a fresh high-nitrate vegetable salad twice per day than after consuming a canned vegetable medley twice per day. As outlined in table 4, subjects had lower peripheral diastolic blood pressure after consuming a fresh high-nitrate vegetable salad twice per day (*pre-intervention* = 72.8 ± 5.3 mm Hg; *post-intervention* = 71.7 ± 5.4 mm Hg) than after consuming a canned vegetable medley twice per day (*pre-control* = 76.6 ± 4.0 mm Hg; *post-control* = 77.5 ± 6.2 mm Hg). When comparing the effects of the high-nitrate and the low-nitrate conditions, there were no statistically significant differences in peripheral diastolic blood pressure (p = 0.684). Given these preliminary findings, the hypothesis was not supported.

Table 4. A comparison of peripheral diastolic blood pressure between the high and low nitrate phases of the study

	High Nitrate	Low Nitrate	P-value	Effect Size
Peripheral DBP (mm Hg)				
Pre	72.8 ± 5.3	76.6 ± 4.0		
Post	71.7 ± 5.4	77.5 ± 6.2		
Δ	-1.1 ± 4.8	0.9 ± 5.3	0.684	0.128

These data were based on n=5 subjects. Statistics were performed using SPSS Statistical Analysis system 23.0. All values represent mean ± Standard Deviation (SD). Data are considered statistically significant at p-value < 0.05.

Central-aortic Systolic Blood Pressure

It was hypothesized that subjects would have lower central-aortic systolic blood pressure after consuming a fresh high-nitrate vegetable salad twice per day than after consuming a canned vegetable medley twice per day. As outlined in table 5, subjects had lower central-aortic systolic blood pressure after consuming a fresh high-nitrate vegetable salad twice per day (*pre-intervention* = 111.8 ± 8.8 mm Hg; *post-intervention* = 109.9 ± 8.7 mm Hg) than after consuming a canned vegetable medley twice per day (*pre-control* = 115.9 ± 5.8 mm Hg; *post-control* = 119.7 ± 11.6 mm Hg). When comparing the effects of the high-nitrate and the low-nitrate conditions, there were no statistically significant differences in central-aortic systolic blood pressure ($p = 0.225$). Given these preliminary findings, the hypothesis was not supported.

Table 5. A comparison of central-aortic systolic blood pressure between the high and low nitrate phases of the study

	High Nitrate	Low Nitrate	P-value	Effect Size
Central SBP (mm Hg)				
Pre	111.8 ± 8.8	115.9 ± 5.8		
Post	109.9 ± 8.7	119.7 ± 11.6		
Δ	-1.9 ± 4.5	3.8 ± 8.1	0.225	0.384

These data were based on n=5 subjects. Statistics were performed using SPSS Statistical Analysis system 23.0. All values represent mean ± Standard Deviation (SD). Data are considered statistically significant at p-value < 0.05.

Central-aortic Diastolic Blood Pressure

It was hypothesized that subjects would have lower central-aortic diastolic blood pressure after consuming a fresh high-nitrate vegetable salad twice per day than after consuming a canned vegetable medley twice per day. As outlined in table 6, subjects had lower central-aortic diastolic blood pressure after consuming a fresh high-nitrate vegetable salad twice per day (*pre-intervention* = 73.8 ± 5.7 mm Hg; *post-intervention* = 72.4 ± 5.4 mm Hg) than after consuming a canned vegetable medley twice per day (*pre-control* = 77.4 ± 4.5 mm Hg; *post-control* = 78.3 ± 6.4 mm Hg). When comparing the effects of the high-nitrate and the low-nitrate conditions, there were no statistically significant differences in central-aortic diastolic blood pressure ($p = 0.465$). Given these preliminary findings, the hypothesis was not supported.

Table 6. A comparison of central-aortic diastolic blood pressure between the high and low nitrate phases of the study

	High Nitrate	Low Nitrate	P-value	Effect Size
Central DBP (mm Hg)				
Pre	73.8 ± 5.7	77.4 ± 4.5		
Post	72.4 ± 5.4	78.3 ± 6.4		
Δ	-1.4 ± 4.5	0.9 ± 4.8	0.465	0.231

These data were based on n=5 subjects. Statistics were performed using SPSS Statistical Analysis system 23.0. All values represent mean ± Standard Deviation (SD). Data are considered statistically significant at p-value < 0.05.

Pulse Wave Velocity

It was hypothesized that subjects would have lower (improved) pulse wave velocity after consuming a fresh high-nitrate vegetable salad twice per day than after consuming a canned vegetable medley twice per day. As outlined in table 7, pulse wave velocity was not significantly different ($p = 0.465$) in subjects after consuming a fresh high-nitrate vegetable salad twice per day (*pre-intervention* = 7.5 ± 0.9 min/sec; *post-*

intervention = 7.6 ± 0.1 min/sec) than after consuming a canned vegetable medley twice per day (*pre-control* = 8.1 ± 1.6 min/sec; *post-control* = 7.7 ± 1.0 min/sec). Given these preliminary findings, the hypothesis was not supported.

Table 7. A comparison of pulse wave velocity between the high and low nitrate phases of the study

	High Nitrate	Low Nitrate	P-value	Effect Size
Pulse wave velocity (min/sec)				
Pre	7.5 ± 0.9	8.1 ± 1.6		
Post	7.6 ± 1.0	7.7 ± 1.0		
Δ	0.1 ± 0.6	-0.4 ± 1.7	0.465	0.231

These data were based on n=5 subjects. Statistics were performed using SPSS Statistical Analysis system 23.0. All values represent mean ± Standard Deviation (SD). Data are considered statistically significant at p-value < 0.05.

Flow-mediated Dilation

It was hypothesized that subjects would have higher (improved) flow-mediated dilation after consuming a fresh high-nitrate vegetable salad twice per day than after consuming a canned vegetable medley twice per day. As outlined in table 8, subjects had higher (improved) flow-mediated dilation after consuming a fresh high-nitrate vegetable salad twice per day (*pre-intervention* = 6.9 ± 2.9 %; *post-intervention* = 8.8 ± 3.6 %) than after consuming a canned vegetable medley twice per day (*pre-control* = 6.9 ± 4.2 %; *post-control* = 5.2 ± 2.9 %). When comparing the effects of the high-nitrate and the low-nitrate conditions, the difference in flow-mediated dilation tended to approach statistical significance (p = 0.080). Given these preliminary findings, the hypothesis was not supported.

Table 8. A comparison of flow-mediated dilation between the high and low nitrate phases of the study

	High Nitrate	Low Nitrate	P-value	Effect Size
Flow-mediated dilation (%)				
Pre	6.9 ± 2.9	6.9 ± 4.2		
Post	8.8 ± 3.6	5.2 ± 2.9		
Δ	1.9 ± 5.0	-1.7 ± 1.8	0.080	0.554

These data were based on n=5 subjects. Statistics were performed using SPSS Statistical Analysis system 23.0. All values represent mean ± Standard Deviation (SD). Data are considered statistically significant at p-value < 0.05.

CHAPTER 5

DISCUSSION

This 8-week randomized, placebo-controlled, crossover trial sought to compare the effects of a fresh, high-nitrate vegetable salad versus a canned, low-nitrate vegetable medley on plasma nitrate/nitrite concentration, peripheral blood pressure, central-aortic blood pressure, pulse wave velocity, and flow-mediated dilation in healthy post-menopausal women. Non-parametric Wilcoxon Signed-Rank tests compared mean differences between conditions. In alignment with the crossover design protocol, all subjects underwent both the intervention and the control conditions of the trial and adhered to a two to three week washout period between feeding phases. Following an extensive literature review, several research hypotheses were developed for this trial.

Plasma Nitrate/Nitrite Concentration. As hypothesized, there was a statistically significant increase in plasma nitrate/nitrite concentration following the high-nitrate intervention versus the low-nitrate condition. These findings are in alignment with previous research in which subjects ingested a high-nitrate intervention. For instance, a randomized crossover study, which sought to assess the short-term effects of green leafy vegetables on blood pressure and arterial stiffness in a sample of subjects with high-normal blood pressure observed a statistically significant increase in salivary and plasma nitrate and nitrite concentration.[71] In a similar study, daily consumption of 250 ml of beetroot juice over a two-week period resulted in significant increases in plasma nitrate/nitrite concentration.[11]

Peripheral and Central-aortic Blood Pressures. Following the 8-week trial, there were no statistically significant differences in peripheral and central-aortic systolic or diastolic

blood pressures between the treatment and control conditions. These findings suggest that consumption of a high-nitrate vegetable salad (≥ 250 mg/100 g fresh weight) did not offer significant antihypertensive benefits. Findings from this study do not align with previous research. A systematic review and meta-analysis of the effects of inorganic nitrate and beetroot juice supplementation revealed that nitrate supplementation was effective for reducing blood pressure in adult populations.[9] Similarly, a crossover trial assessing the intake of supplementary beetroot juice administered to healthy young subjects demonstrated a significant reduction of approximately 4% in both systolic and diastolic blood pressure which was maintained for roughly two weeks with continued administration of the intervention.[95] In other studies, consumption of high-nitrate food or beverage items resulted in an increased bioactivation of nitric oxide which led to vasoprotective outcomes in as little as three hours after ingestion.[95,97] However, to the best of the investigators' knowledge, no trials have assessed the effectiveness of fresh, high-nitrate vegetable salads on blood pressure in post-menopausal women. Further, it is important to note that the present study was underpowered as a result of the small sample of study participants.

Pulse Wave Velocity. Contrary to the researcher's hypothesis, there were no statistically significant improvements in pulse wave velocity following the high-nitrate intervention versus the low-nitrate control condition. It is important to note that previous research has found that post-menopausal women generally have higher pulse wave velocity levels than menstruating women.[75] To date, no known studies have exclusively assessed the effectiveness of dietary nitrates on pulse wave velocity in post-menopausal women. The few studies which have examined the effectiveness of dietary nitrates on pulse wave velocity have included men and women with regular menses.[15,141,142] In particular, a

randomized, double-blind crossover trial conducted on healthy adults revealed that supplementation of inorganic nitrate resulted in a significant decrease (improvement) in pulse wave velocity.[15,141] Conversely, another randomized-controlled crossover trial in which high-nitrate spinach was supplemented did not show any significant reductions (improvements) in pulse wave velocity measurements.[142]

Flow-mediated Dilation. The investigators hypothesized that flow-mediation would increase (improve) following the high-nitrate intervention versus the low-nitrate control condition. Findings suggest partial support for this hypothesis with an effect size that indicates that the trial was just underpowered to see an effect of the high-nitrate intervention. To date, no known studies have exclusively assessed the effectiveness of dietary nitrates on flow-mediated dilation in post-menopausal women. The few studies which have examined the effectiveness of dietary nitrates on flow-mediated dilation have shown mixed results. In particular, a study conducted on healthy adults revealed that supplementation of inorganic nitrate did not result in a significant increase (improvement) in flow-mediated dilation.[141] Conversely, a randomized-controlled crossover trial examining the effects of flavonoid-rich apples and high-nitrate spinach in healthy adult men and women resulted in a significant increase (improvement) in flow-mediated dilation.[15,141]

Strengths. This randomized, placebo-controlled trial had several notable strengths. First, findings indicate that the high-nitrate intervention had a sustained effect on plasma nitrate/nitrite concentration given that there was a statistically significant improvement in plasma nitrate/nitrite concentration even after a 12-hour overnight fast. Next, the study's crossover design allowed the researchers to assess within-subject changes following the

control and intervention phases of the trial. By utilizing a crossover design as well as instructing subjects to observe a pre-determined washout phase, the researchers attained a comparable level of statistical power as with a parallel arm trial – this was especially appropriate given the small sample size of this trial. Based on fidelity calendars, there was a 98% overall compliance for consumption of the high-nitrate salads and a 90% overall compliance for consumption of the low-nitrate canned vegetables. Other strengths are that subjects were asked to refrain from multivitamins, antihistamines, and decongestants three days prior to study visits two through five, and from alcoholic beverages, caffeine, and heavy exercise 12-hours prior to study visits two through five. Additionally, subjects on antihypertensive medications and those taking antiseptic/antibacterial mouth rinses were excluded from the trial. Researchers concur that consumption of caffeine and antiseptic/antibacterial mouth rinses may interfere with blood pressure readings.[105, 131]

Limitations. As with all research, there were inherent limitations to this trial. Despite the goal of enrolling 15 subjects, only 5 subjects completed the trial. Given the limited sample size, this trial may have lacked the power to detect statistically significant effects. Yet another limitation of this trial was that the actual nitrate levels present in the vegetable salads were unknown. Future research seeking to assess the effectiveness of a high-nitrate vegetable salad on cardiovascular parameters in post-menopausal subjects may benefit from obtaining vegetables from a single grower or collecting and testing nitrate levels in soil samples in which vegetables were obtained, analyzing the nitrate content in vegetables once procured, weighing ingredients, preparing salads for subjects,

ensuring that salads are consumed in the presence of the investigators, and recruiting a larger sample of subjects.

Conclusion. This 8-week randomized, placebo-controlled, crossover trial suggests that twice daily consumption of a high-nitrate vegetable salad (≥ 250 mg/100 g fresh weight) does not significantly decrease peripheral systolic or diastolic blood pressure, central-aortic systolic or diastolic blood pressure or improve flow-mediated dilation or pulse wave velocity when compared to the low-nitrate condition. Conversely, when compared to the low-nitrate condition, twice daily consumption of a high nitrate vegetable salad did significantly increase plasma nitrate/nitrite concentration.

REFERENCES

1. Mozaffarian D, Benjamin EJ, Go AS, et al. Heart Disease and Stroke Statistics—2015 Update: A Report From the American Heart Association. *Circulation.* 2015;131(4):e29-e322.

2. Centers for Disease Control and Prevention. High Blood Pressure Facts. 2015; http://www.cdc.gov/bloodpressure/facts.htm. Accessed 27 January, 2016.

3. Carretero OA, Oparil S. Essential hypertension part I: definition and etiology. *Circulation.* 2000;101(3):329-335.

4. Cutler J. High blood pressure and end-organ damage. *Journal of hypertension. Supplement: official journal of the International Society of Hypertension.* 1996;14(6):S3-6.

5. Appel LJ, Brands MW, Daniels SR, Karanja N, Elmer PJ, Sacks FM. Dietary approaches to prevent and treat hypertension a scientific statement from the American Heart Association. *Hypertension.* 2006;47(2):296-308.

6. Martins D, Nelson K, Pan D, Tareen N, Norris K. The effect of gender on age-related blood pressure changes and the prevalence of isolated systolic hypertension among older adults: data from NHANES III. *The journal of gender-specific medicine: JGSM: the official journal of the Partnership for Women's Health at Columbia.* 2000;4(3):10-13, 20.

7. Sacks FM, Svetkey LP, Vollmer WM, et al. Effects on Blood Pressure of Reduced Dietary Sodium and the Dietary Approaches to Stop Hypertension (DASH) Diet. *The New England Journal of Medicine.* 2001;344(1):3-10.

8. Salehi-Abargouei A, Maghsoudi Z, Shirani F, Azadbakht L. Effects of Dietary Approaches to Stop Hypertension (DASH)-style diet on fatal or nonfatal cardiovascular diseases--incidence: a systematic review and meta-analysis on observational prospective studies. *Nutrition (Burbank, Los Angeles County, Calif.).* 2013;29(4):611-618.

9. Siervo M, Lara J, Ogbonmwan I, Mathers JC. Inorganic nitrate and beetroot juice supplementation reduces blood pressure in adults: a systematic review and meta-analysis. *The Journal of nutrition.* 2013;143(6):818.

10. Bondonno CP, Liu AH, Croft KD, et al. Absence of an effect of high nitrate intake from beetroot juice on blood pressure in treated hypertensive individuals: a randomized controlled trial. *The American journal of clinical nutrition.* 2015;102(2):368-375.

11. Gilchrist M, Winyard PG, Aizawa K, Anning C, Shore A, Benjamin N. Effect of dietary nitrate on blood pressure, endothelial function, and insulin sensitivity in type 2 diabetes. *Free Radical Biology and Medicine.* 2013;60:89-97.

12. Kelly J, Fulford J, Vanhatalo A, et al. Effects of short-term dietary nitrate supplementation on blood pressure, O2 uptake kinetics, and muscle and cognitive function in older adults. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology.* 2013;304(2):R73-R83.

13. Jajja A, Sutyarjoko A, Lara J, et al. Beetroot supplementation lowers daily systolic blood pressure in older, overweight subjects. *Nutrition research (New York, N.Y.).* 2014;34(10):868-875.

14. Kapil V, Khambata RS, Robertson A, Caulfield MJ, Ahluwalia A. Dietary nitrate provides sustained blood pressure lowering in hypertensive patients a randomized, phase 2, double-blind, placebo-controlled study. *Hypertension.* 2015;65(2):320-327.

15. Bondonno CP, Croft KD, Ward N, Considine MJ, Hodgson JM. Dietary flavonoids and nitrate: effects on nitric oxide and vascular function. *Nutrition Reviews.* 2015;73(4):216-235.

16. Miller GD, Marsh AP, Dove RW, et al. Plasma nitrate and nitrite are increased by a high-nitrate supplement but not by high-nitrate foods in older adults. *Nutrition Research.* 2012;32(3):160-168.

17. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nature Reviews Drug Discovery.* 2008;7(2):156-167.

18. Nwankwo T, Yoon SS, Burt V, Gu Q. Hypertension among adults in the United States: National Health and Nutrition Examination Survey, 2011-2012. *NCHS data brief.* 2013(133):1-8.

19. National Heart L, and Blood Institute, . Description of High Blood Pressure. 2015; https://www.nhlbi.nih.gov/health/health-topics/topics/hbp. Accessed 2 February, 2016.

20. PubMed Health. High Blood Pressure (Hypertension). http://www.ncbi.nlm.nih.gov/pubmedhealth/PMHT0024199/. Accessed 2 February, 2016.

21. Mayo Clinic. High blood pressure (hypertension). http://www.mayoclinic.org/diseases-conditions/high-blood-pressure/basics/causes/con-20019580. Accessed 5 February, 2016.

22. Gong M, Hubner N. Molecular genetics of human hypertension. *Clinical science.* 2006;110(3):315-326.

23. Node K, Kitakaze M, Yoshikawa H, Kosaka H, Hori M. Reduced plasma concentrations of nitrogen oxide in individuals with essential hypertension. *Hypertension.* 1997;30(3):405-408.

24. Grotto I, Huerta M, Sharabi Y. Hypertension and socioeconomic status. *Current Opinion in Cardiology.* 2008;23(4):335-339.

25. Fan AZ, Strasser SM, Zhang X, Fang J, Crawford CG. State socioeconomic indicators and self-reported hypertension among US adults, 2011 behavioral risk factor surveillance system. *Preventing chronic disease U6 - ctx_ver=Z39.88-2004&ctx_enc=info%3Aofi%2Fenc%3AUTF-8&rfr_id=info:sid/summon.serialssolutions.com&rft_val_fmt=info:ofi/fmt:kev:mtx:journal&rft.genre=article&rft.atitle=State+socioeconomic+indicators+and+self-reported+hypertension+among+US+adults%2C+2011+behavioral+risk+factor+surveillance+system&rft.jtitle=Preventing+chronic+disease&rft.au=Fan%2C+Amy+Z&rft.au=Strasser%2C+Sheryl+M&rft.au=Zhang%2C+Xingyou&rft.au=Fang%2C+Jing&rft.date=2015&rft.eissn=1545-1151&rft.volume=12&rft.spage=E27&rft_id=info:pmid/25719217&rft.externalDocID=25719217&paramdict=en-US U7 - Journal Article.* 2015;12:E27.

26. Rosano G, Vitale C, Marazzi G, Volterrani M. Menopause and cardiovascular disease: the evidence. *Climacteric.* 2007;10(sup1):19-24.

27. Epstein FH, Wei JY. Age and the cardiovascular system. *New England Journal of Medicine.* 1992;327(24):1735-1739.

28. Centers for Disease Control and Prevention. Hypertension among adults in the United States, 2009–2010. 2012; http://www.cdc.gov/nchs/data/databriefs/db107.htm. Accessed 5 February, 2016.

29. Maillard P, Seshadri S, Beiser A, et al. Effects of systolic blood pressure on white-matter integrity in young adults in the Framingham Heart Study: a cross-sectional study. *The Lancet Neurology.* 2012;11(12):1039-1047.

30. Obisesan TO, Obisesan OA, Martins S, et al. High blood pressure, hypertension, and high pulse pressure are associated with poorer cognitive function in persons aged 60 and older: the Third National Health and Nutrition Examination Survey. *Journal of the American Geriatrics Society.* 2008;56(3):501-509.

31. Schilling R. High Blood Pressure Decreases Cognitive Function. 2008.

32. Nagai M, Hoshide S, Ishikawa J, Shimada K, Kario K. Ambulatory blood pressure as an independent determinant of brain atrophy and cognitive function in elderly hypertension. *Journal of hypertension.* 2008;26(8):1636-1641.

33. Gambacciani M, Ciaponi M, Cappagli B, De Simone L, Orlandi R, Genazzani A. Prospective evaluation of body weight and body fat distribution in early postmenopausal women with and without hormonal replacement therapy. *Maturitas.* 2001;39(2):125-132.

34. Committee G. 2003 European Society of Hypertension–European Society of Cardiology guidelines for the management of arterial hypertension*. *Journal of hypertension.* 2003;21(6):1011-1053.

35. Wilson PW, Kannel WB. Epidemiology of hyperglycemia and atherosclerosis. *Hyperglycemia, diabetes, and vascular disease*: Springer; 1992:21-29.

36. Group DS. Prediction of the risk of cardiovascular mortality using a score that includes glucose as a risk factor. The DECODE Study. *Diabetologia.* 2004;47(12):2118-2128.

37. World Health Organization. Cardiovascular Diseases (CVDs). http://www.who.int/mediacentre/factsheets/fs317/en/. Accessed 5 May, 2016.

38. Kannel WB, Wolf PA, McGee DL, Dawber TR, McNamara P, Castelli WP. Systolic blood pressure, arterial rigidity, and risk of stroke: the Framingham study. *Jama.* 1981;245(12):1225-1229.

39. Dubow J, Fink ME. Impact of hypertension on stroke. *Current atherosclerosis reports.* 2011;13(4):298-305.

40. Kannel WB, Wolf PA, Verter J, McNamara PM. Epidemiologic assessment of the role of blood pressure in stroke: the Framingham study. *Jama.* 1970;214(2):301-310.

41. Kario K, Ishikawa J, Pickering TG, et al. Morning hypertension: the strongest independent risk factor for stroke in elderly hypertensive patients. *Hypertension research.* 2006;29(8):581.

42. Ritz E. Hypertension and kidney disease. *Clinical nephrology.* 2010;74:S39-43.

43. Bakris GL, Ritz E. The message for World Kidney Day 2009: hypertension and kidney disease: a marriage that should be prevented. *The Journal of Clinical Hypertension.* 2009;11(3):144-147.

44. Stoner L, Young JM, Fryer S. Assessments of arterial stiffness and endothelial function using pulse wave analysis. *International journal of vascular medicine.* 2012;2012.

45. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *Journal of the American College of Cardiology.* 2002;39(2):257-265.

46. Feletou M. Part 1: Multiple Functions of the Endothelial Cells-Focus on Endothelium-Derived Vasoactive Mediators. *The endothelium. Morgan and Claypool Life Sciences, San Rafael.* 2011.

47. Uehata A, Lieberman E, Gerhard M, et al. Noninvasive assessment of endothelium-dependent flow-mediated dilation of the brachial artery. *Vascular medicine (London, England).* 1996;2(2):87-92.

48. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation.* 2004;109(23 suppl 1):III-27-III-32.

49. Moens AL, Goovaerts I, Claeys MJ, Vrints CJ. Flow-mediated vasodilation: a diagnostic instrument, or an experimental tool? *CHEST Journal.* 2005;127(6):2254-2263.

50. Tousoulis D, Davies G, Crake T. Acetylcholine and endothelial function. *Circulation.* 1998;98(15):1587a-1590.

51. Harris RA, Nishiyama SK, Wray DW, Richardson RS. Ultrasound assessment of flow-mediated dilation. *Hypertension.* 2010;55(5):1075-1085.

52. Flammer AJ, Anderson T, Celermajer DS, et al. The assessment of endothelial function from research into clinical practice. *Circulation.* 2012;126(6):753-767.

53. Benjamin EJ, Larson MG, Keyes MJ, et al. Clinical Correlates and Heritability of Flow-Mediated Dilation in the Community The Framingham Heart Study. *Circulation.* 2004;109(5):613-619.

54. Yavuz B, Yavuz B, Sener DD, et al. Advanced age is associated with endothelial dysfunction in healthy elderly subjects. *Gerontology.* 2008;54(3):153-156.

55. Skaug E-A, Aspenes ST, Oldervoll L, et al. Age and gender differences of endothelial function in 4739 healthy adults: the HUNT3 Fitness Study. *European journal of preventive cardiology.* 2013;20(4):531-540.

56. Cortés B, Núñez I, Cofán M, et al. Acute effects of high-fat meals enriched with walnuts or olive oil on postprandial endothelial function. *Journal of the American College of Cardiology.* 2006;48(8):1666-1671.

57. Fan Z, Zhang G, Liao S. Pulse wave analysis. *Advanced Biomedical Engineering.* 2011:21-40.

58. Hirata K, Kawakami M, O'Rourke MF. Pulse wave analysis and pulse wave velocity a review of blood pressure interpretation 100 years after Korotkov. *Circulation journal.* 2006;70(10):1231-1239.

59. O'Rourke MF, Gallagher DE. Pulse wave analysis. *JOURNAL OF HYPERTENSION-SUPPLEMENT-.* 1996;14:S147-S158.

60. Payne RA, Wilkinson IB, Webb DJ. Arterial stiffness and hypertension emerging concepts. *Hypertension.* 2010;55(1):9-14.

61. Scuteri A, Cunha PG, Rosei EA, et al. Arterial stiffness and influences of the metabolic syndrome: a cross-countries study. *Atherosclerosis.* 2014;233(2):654-660.

62. Vlachopoulos C, Kosmopoulou F, Alexopoulos N, Ioakeimidis N, Siasos G, Stefanadis C. Acute mental stress has a prolonged unfavorable effect on arterial stiffness and wave reflections. *Psychosomatic medicine.* 2006;68(2):231-237.

63. Vlachopoulos C, Xaplanteris P, Alexopoulos N, et al. Divergent effects of laughter and mental stress on arterial stiffness and central hemodynamics. *Psychosomatic medicine.* 2009;71(4):446-453.

64. Mattace-Raso FU, van der Cammen TJ, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke the rotterdam study. *Circulation.* 2006;113(5):657-663.

65. Leoncini G, Ratto E, Viazzi F, et al. Increased ambulatory arterial stiffness index is associated with target organ damage in primary hypertension. *Hypertension.* 2006;48(3):397-403.

66. Jatoi NA, Jerrard-Dunne P, Feely J, Mahmud A. Impact of smoking and smoking cessation on arterial stiffness and aortic wave reflection in hypertension. *Hypertension.* 2007;49(5):981-985.

67. Collaboration RVfAS. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *European heart journal.* 2010;31(19):2338-2350.

68. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *European heart journal.* 2006;27(21):2588-2605.

69. Choi CU, Park EB, Suh SY, et al. Impact of Aortic Stiffness on Cardiovascular Disease in Patients With Chest Pain* Assessment With Direct Intra-Arterial Measurement. *American journal of hypertension.* 2007;20(11):1163-1169.

70. Shokawa T, Imazu M, Yamamoto H, et al. Pulse wave velocity predicts cardiovascular mortality-findings from the Hawaii-Los Angeles-Hiroshima study. *Circulation Journal.* 2005;69(3):259-264.

71. Bondonno CP, Liu AH, Croft KD, et al. Short-term effects of nitrate-rich green leafy vegetables on blood pressure and arterial stiffness in individuals with high-normal blood pressure. *Free radical biology & medicine.* 2014;77:353-362.

72. Lebrun CE, van der Schouw YT, Bak AA, et al. Arterial stiffness in postmenopausal women: determinants of pulse wave velocity. *Journal of hypertension.* 2002;20(11):2165-2172.

73. Tomiyama H, Yamashina A, Arai T, et al. Influences of age and gender on results of noninvasive brachial–ankle pulse wave velocity measurement—a survey of 12 517 subjects. *Atherosclerosis.* 2003;166(2):303-309.

74. Maiello M, Zito A, Ciccone MM, Palmiero P. How Aortic Stiffness in Postmenopausal Women Is Related to Common Cardiovascular Risk Factors. *Cardiology research and practice.* 2014;2014.

75. Palmiero P, Maiello M, Daly DD, Ciccone MM, Nanda NC. Aortic stiffness assessed by global pulse wave velocity in postmenopausal women: an ultrasonographic study. *Echocardiography.* 2012;29(10):1233-1238.

76. Zhou L, Zhu D-Y. Neuronal nitric oxide synthase: structure, subcellular localization, regulation, and clinical implications. *Nitric Oxide.* 2009;20(4):223-230.

77. Napoli C, de Nigris F, Williams-Ignarro S, Pignalosa O, Sica V, Ignarro LJ. Nitric oxide and atherosclerosis: an update. *Nitric oxide.* 2006;15(4):265-279.

78. Kelm M. The L-arginine-nitric oxide pathway in hypertension. *Current hypertension reports.* 2003;5(1):80-86.

79. Taddei S, Virdis A, Ghiadoni L, Salvetti A. Endothelial dysfunction in hypertension: fact or fancy? *Journal of cardiovascular pharmacology.* 1997;32:S41-47.

80. Kelm M, Preik M, Hafner DJ, Strauer BE. Evidence for a multifactorial process involved in the impaired flow response to nitric oxide in hypertensive patients with endothelial dysfunction. *Hypertension.* 1996;27(3):346-353.

81. Feelisch M, Kelm M. Biotransformation of organic nitrates to nitric oxide by vascular smooth muscle and endothelial cells. *Biochemical and biophysical research communications.* 1991;180(1):286-293.

82. Shiva S, Wang X, Ringwood LA, et al. Ceruloplasmin is a NO oxidase and nitrite synthase that determines endocrine NO homeostasis. *Nature chemical biology.* 2006;2(9):486-493.

83. Ignarro L. Nitric oxide as a unique signaling molecule in the. *Journal of physiology and pharmacology.* 2002;53(4):503-514.

84. Cosby K, Partovi KS, Crawford JH, et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nature medicine.* 2003;9(12):1498-1505.

85. Shiva S, Huang Z, Grubina R, et al. Deoxymyoglobin is a nitrite reductase that generates nitric oxide and regulates mitochondrial respiration. *Circulation research.* 2007;100(5):654-661.

86. Duncan C, Dougall H, Johnston P, et al. Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. *Nature medicine.* 1995;1(6):546-551.

87. Benjamin N, O'Driscoll F, Dougall H, et al. Stomach NO synthesis. 1994.

88. Lundberg J, Weitzberg E, Lundberg J, Alving K. Intragastric nitric oxide production in humans: measurements in expelled air. *Gut.* 1994;35(11):1543-1546.

89. Santamaria P. Nitrate in vegetables: toxicity, content, intake and EC regulation. *Journal of the Science of Food and Agriculture.* 2006;86(1):10-17.

90. Kleinbongard P, Dejam A, Lauer T, et al. Plasma nitrite concentrations reflect the degree of endothelial dysfunction in humans. *Free Radical Biology and Medicine.* 2006;40(2):295-302.

91. Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Vol 90. United States: American Society for Clinical Nutrition, Inc; 2009:1-10.

92. Most MM. Estimated phytochemical content of the dietary approaches to stop hypertension (DASH) diet is higher than in the Control Study Diet. *Journal of the American Dietetic Association.* 2004;104(11):1725-1727.

93. Lundberg JO, Weitzberg E. NO generation from inorganic nitrate and nitrite: role in physiology, nutrition and therapeutics. *Archives of pharmacal research.* 2009;32(8):1119-1126.

94. Hishikawa K, Nakaki T, Suzuki H, Katot R, Saruta T. Role of L-arginine-nitric oxide pathway in hypertension. *Journal of hypertension.* 1993;11(6):639-645.

95. Vanhatalo A, Bailey SJ, Blackwell JR, et al. Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology.* 2010;299(4):R1121-R1131.

96. Jonvik KL, Nyakayiru J, Pinckaers PJ, Senden JM, van Loon LJ, Verdijk LB. Nitrate-Rich Vegetables Increase Plasma Nitrate and Nitrite Concentrations and Lower Blood Pressure in Healthy Adults. *The Journal of nutrition.* 2016;146(5):986-993.

97. Webb AJ, Patel N, Loukogeorgakis S, et al. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension.* 2008;51(3):784-790.

98. Cantliffe D. Nitrate accumulation in table beets and spinach as affected by nitrogen, phosphorus, and potassium nutrition and light intensity. *Agronomy Journal.* 1973;65(4):563-565.

99. Santamaria P, Elia A, Gonnella M. Changes in nitrate accumulation and growth of endive plants during light period as affected by nitrogen level and form 1. *Journal of plant nutrition.* 1997;20(10):1255-1266.

100. Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of dietary nitrate on blood pressure in healthy volunteers. *New England Journal of Medicine.* 2006;355(26):2792-2793.

101. van Velzen AG, Sips AJ, Schothorst RC, Lambers AC, Meulenbelt J. The oral bioavailability of nitrate from nitrate-rich vegetables in humans. *Toxicology letters.* 2008;181(3):177-181.

102. Tannenbaum S, Weisman M, Fett D. The effect of nitrate intake on nitrite formation in human saliva. *Food and cosmetics toxicology.* 1976;14(6):549-552.

103. Woessner M, Smoliga JM, Tarzia B, Stabler T, Van Bruggen M, Allen JD. A stepwise reduction in plasma and salivary nitrite with increasing strengths of mouthwash following a dietary nitrate load. *Nitric Oxide.* 2016.

104. McDonagh S, Wylie L, Winyard P, Vanhatalo A, Jones A. The Effects of Chronic Nitrate Supplementation and the Use of Strong and Weak Antibacterial Agents on Plasma Nitrite Concentration and Exercise Blood Pressure. *International journal of sports medicine.* 2015;36(14):1177-1185.

105. Bondonno CP, Liu AH, Croft KD, et al. Antibacterial mouthwash blunts oral nitrate reduction and increases blood pressure in treated hypertensive men and women. *American journal of hypertension.* 2014:hpu192.

106. Persson MG, Gustafsson LE. Ethanol can inhibit nitric oxide production. *European journal of pharmacology.* 1992;224(1):99-100.

107. Puddey I, Zilkens R, Croft K, Beilin L. Alcohol and endothelial function: a brief review. *Clinical and experimental pharmacology and physiology.* 2001;28(12):1020-1024.

108. Romelsjö A, Leifman A. Association between alcohol consumption and mortality, myocardial infarction, and stroke in 25 year follow up of 49 618 young Swedish men. *Bmj.* 1999;319(7213):821-822.

109. Chobanian AV, Bakris GL, Black HR, et al. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. *Jama.* 2003;289(19):2560-2571.

110. Warburton DE, Nicol CW, Bredin SS. Health benefits of physical activity: the evidence. *Canadian medical association journal.* 2006;174(6):801-809.

111. Hu FB, Willett WC, Li T, Stampfer MJ, Colditz GA, Manson JE. Adiposity as compared with physical activity in predicting mortality among women. *New England Journal of Medicine.* 2004;351(26):2694-2703.

112. Myers J, Kaykha A, George S, et al. Fitness versus physical activity patterns in predicting mortality in men. *The American journal of medicine.* 2004;117(12):912-918.

113. Blair SN. Physical inactivity: the biggest public health problem of the 21st century. *British journal of sports medicine.* 2009;43(1):1-2.

114. Church TS, Earnest CP, Skinner JS, Blair SN. Effects of different doses of physical activity on cardiorespiratory fitness among sedentary, overweight or obese postmenopausal women with elevated blood pressure: a randomized controlled trial. *Jama.* 2007;297(19):2081-2091.

115. Blumenthal JA, Babyak MA, Hinderliter A, et al. Effects of the DASH diet alone and in combination with exercise and weight loss on blood pressure and cardiovascular biomarkers in men and women with high blood pressure: the ENCORE study. *Archives of internal medicine.* 2010;170(2):126-135.

116. Fagard R. Exercise is good for your blood pressure: effects of endurance training and resistance training. *Clinical and Experimental Pharmacology and Physiology.* 2006;33(9):853-856.

117. Fagard RH, Cornelissen VA. Effect of exercise on blood pressure control in hypertensive patients. *European Journal of Cardiovascular Prevention & Rehabilitation.* 2007;14(1):12-17.

118. Krumholz H, Ross JS, Presler AH, Egilman DS. What have we learnt from Vioxx? *British Medical Journal.* 2007;7585:120.

119. Antman EM, Bennett JS, Daugherty A, Furberg C, Roberts H, Taubert KA. Use of nonsteroidal antiinflammatory drugs an update for clinicians: a scientific statement from the American Heart Association. *Circulation.* 2007;115(12):1634-1642.

120. Salerno SM, Jackson JL, Berbano EP. Effect of oral pseudoephedrine on blood pressure and heart rate: a meta-analysis. *Archives of internal medicine.* 2005;165(15):1686-1694.

121. Whelton PK, He J, Cutler JA, et al. Effects of oral potassium on blood pressure: meta-analysis of randomized controlled clinical trials. *Jama.* 1997;277(20):1624-1632.

122. Giebisch G. Renal potassium transport: mechanisms and regulation. *American Journal of Physiology-Renal Physiology.* 1998;274(5):F817-F833.

123. Agarwal R, Afzalpurkar R, Fordtran JS. Pathophysiology of potassium absorption and secretion by the human intestine. *Gastroenterology.* 1994;107(2):548-571.

124. Zhou M-S, Kosaka H, Yoneyama H. Potassium augments vascular relaxation mediated by nitric oxide in the carotid arteries of hypertensive Dahl rats. *American journal of hypertension.* 2000;13(6):666-672.

125. Haddy FJ, Vanhoutte PM, Feletou M. Role of potassium in regulating blood flow and blood pressure. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology.* 2006;290(3):R546-R552.

126. Stolarz-Skrzypek K, Bednarski A, Czarnecka D, Kawecka-Jaszcz K, Staessen JA. Sodium and potassium and the pathogenesis of hypertension. *Current hypertension reports.* 2013;15(2):122-130.

127. Robertson JIS. Dietary salt and hypertension: a scientific issue or a matter of faith? *Journal of evaluation in clinical practice.* 2003;9(1):1-22.

128. Geerling JC, Loewy AD. Central regulation of sodium appetite. *Experimental physiology.* 2008;93(2):177-209.

129. Pimenta E, Gaddam KK, Oparil S, et al. Effects of dietary sodium reduction on blood pressure in subjects with resistant hypertension results from a randomized trial. *Hypertension.* 2009;54(3):475-481.

130. Group ICR. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. *Bmj.* 1988;297:319-328.

131. Noordzij M, Uiterwaal CS, Arends LR, Kok FJ, Grobbee DE, Geleijnse JM. Blood pressure response to chronic intake of coffee and caffeine: a meta-analysis of randomized controlled trials. *Journal of hypertension.* 2005;23(5):921-928.

132. Sung BH, Lovallo WR, Pincomb GA, Wilson MF. Effects of caffeine on blood pressure response during exercise in normotensive healthy young men. *The American journal of cardiology.* 1990;65(13):909-913.

133. Farag NH, Whitsett TL, McKey BS, et al. Caffeine and blood pressure response: sex, age, and hormonal status. *Journal of Women's Health.* 2010;19(6):1171-1176.

134. Mesas AE, Leon-Muñoz LM, Rodriguez-Artalejo F, Lopez-Garcia E. The effect of coffee on blood pressure and cardiovascular disease in hypertensive individuals: a systematic review and meta-analysis. *The American journal of clinical nutrition.* 2011:ajcn. 016667.

135. Juraschek SP, Guallar E, Appel LJ, Miller ER. Effects of vitamin C supplementation on blood pressure: a meta-analysis of randomized controlled trials. *The American journal of clinical nutrition.* 2012;95(5):1079-1088.

136. Hoitink A. Research on the influence of vitamin C administration on the human organism, in particular in connection with the working capacity. *Verh Nederlands Inst Praevent.* 1946;4:62-63.

137. Moran JP, Cohen L, Greene J, et al. Plasma ascorbic acid concentrations relate inversely to blood pressure in human subjects. *The American journal of clinical nutrition.* 1993;57(2):213-217.

138. Rodrigo R, Prat H, Passalacqua W, Araya J, Bächler JP. Decrease in oxidative stress through supplementation of vitamins C and E is associated with a reduction in blood pressure in patients with essential hypertension. *Clinical Science.* 2008;114(10):625-634.

139. Fotherby MD, Williams JC, Forster LA, Craner P, Ferns GA. Effect of vitamin C on ambulatory blood pressure and plasma lipids in older persons. *Journal of hypertension.* 2000;18(4):411-415.

140. Cutler JA, Sorlie PD, Wolz M, Thom T, Fields LE, Roccella EJ. Trends in hypertension prevalence, awareness, treatment, and control rates in United States adults between 1988–1994 and 1999–2004. *Hypertension.* 2008;52(5):818-827.

141. Bondonno CP, Yang X, Croft KD, et al. Flavonoid-rich apples and nitrate-rich spinach augment nitric oxide status and improve endothelial function in healthy men and women: a randomized controlled trial. *Free Radical Biology and Medicine.* 2012;52(1):95-102.

APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL

APPROVAL: MODIFICATION

Carol Johnston
SNHP: Nutrition
602/827-2265
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:
On 6/22/2016 the ASU IRB reviewed the following protocol:

Type of Review: Modification
Title: A Comparison of a High-Nitrate versus a Low-Nitrate Diet on Cardiovascular Health in Post-Menopausal Women
Investigator: Carol Johnston
IRB ID: STUDY00004354
Funding: Name: Graduate College
Grant Title: None
Grant ID: None
Documents Reviewed:
• screening questionnaire, Category: Screening forms;
• consent, Category: Consent Form;
• data sheet, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);
• research ad, Category: Recruitment Materials;
• calendar, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);
• response to reviewers, Category: IRB Protocol;
• online survey, Category: Recruitment Materials;
• FFQ, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);
• procedure, Category: Technical materials/diagrams;
• protocol,
Category: IRB Protocol;
• FMD data sheet, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);

The IRB approved the modification.
Sincerely,

IRB Administrator

APPENDIX B

CONSENT FORM

ASU School of Nutrition & Health Promotion

CONSENT FORM

Title of research study: A Comparison of a High-Nitrate versus a Low-Nitrate Diet on Cardiovascular Health in Post-Menopausal Women

Investigators: Drs. Karen Sweazea and Carol Johnston, ASU Nutrition Professors

Why am I being invited to take part in a research study?

We invite you to take part in this research study because you are a healthy, post-menopausal woman (40-70 years old) with a blood pressure reading at or above 115/70 mmHg (we will test), and are not currently taking hypertensive medication. You must be willing to participate in an 8-week feeding trial, and willing to eat two vegetable salads or consume a vegetable medley (canned vegetables) daily during the intervention phase of the study.

Why is this research being done?

The purpose of this study is to determine if eating a vegetable salad twice per day improves blood pressure and other cardiovascular parameters in healthy, post-menopausal women (40-70 years old).

How long will the research last?

We expect individuals to spend approximately 8 weeks participating in study activities.

How many people will be studied?

We expect that 15 women will participate in this research study.

What happens if I say yes, I want to be in this research?

You will be a participant in this study for a total of 8 weeks. You will be asked to refrain from:

- Antibacterial mouthwash for the duration of the study.
- Multivitamins/minerals, aspirin, and anti-inflammatory medications for three days prior to blood draws.
- All food and beverages including alcohol and caffeine, smoking, and heavy exercise for 12 hours prior to blood draws. Water will be encouraged during the 12 hour fast.

In total, your participation includes a total of five visits to the research laboratory:

Visit 1: you will go through a consent process, screening questionnaire, food frequency questionnaire, blood pressure measurement. You will also be given a calendar to track your progress (30 minutes)
Visit 2: we will take your blood vessel function and blood pressure measurements. We will also give you a 10-day supply of vegetable salads and salad dressings. If spoilage is a concern, you may choose to visit the laboratory one additional time to pick up additional salads (45-60 minutes)
Visit 3: measurements obtained at visit 2 will be repeated (45-60 minutes)
Visit 4: will be scheduled following the no treatment period (2-2.5 weeks). Here, we will also take your blood vessel function and blood pressure measurements, and will give you canned vegetables for your 10-day treatment (45-60 minutes)
Visit 5: will take place after the 10-day treatment period, and we will once again take your blood vessel function and blood pressure measurements (45-60 minutes)

For the blood sample, you will need to fast overnight for 12 hours (no food or beverage with the exception of water). Approximately 2 tablespoons of blood will be collected by a trained phlebotomist at visits 2, 3, 4, and 5. You will be asked to refrain from all dietary supplements for 3 days prior to testing (e.g., visits 2, 3, and 4); caffeine for 12 hours prior to testing; exercise for 12 hours prior to testing. At visit 2, you will be randomly assigned (by coin toss) to begin either the salad or vegetable medley phase of the study. You will also have a blood pressure cuff inflated on your lower arm, which will be left for 5 minutes then released. Using an ultrasound machine, we will examine your blood vessel function. During the study, you will interact with the research team, consisting of the investigators, a registered nurse, and a sonographer. The contact information of the investigators will be provided to you. All measurements, as well as the processing of the blood sample, will be done at the Arizona Biomedical Collaborative laboratory (ABC) on the ASU downtown campus in Phoenix. The research trial is expected to last from May to August 2016.

ASU IRB IRB # STUDY00004354 | Approval Period 5/17/2016 – 5/16/2017

What happens if I say yes, but I change my mind later?

You may choose to withdraw from this study at any time without penalty or adverse consequence. However, should you decide to leave the study, please inform the investigators of your decision.

Is there any way being in this study could be bad for me?

You may faint or feel temporarily nauseous during the blood draw. All blood draws are performed by a registered nurse, or a trained phlebotomist who are experienced in handling these issues. The measurement of blood vessel function entails placing a blood pressure cuff on your arm and pumping air into the cuff to cut off blood circulation. You will feel pressure on your arm for approximately 5 minutes. In other research, participants ranked the pain associated with this procedure a '2' on a 10-point scale. A trained sonographer (ultrasound technician) will perform this procedure. Taking part in this research study may result in added costs and time commitments. You may need to pay the city meter for curbside parking (approximately $1.50 per visit). The length of each visit should be less than 60 minutes.

Will being in this study help me in any way?

Participating in this study will help advance knowledge on how vegetable salads can improve cardiovascular health. However, we cannot promise any benefits to you or others from your taking part in this research.

What happens to the information collected for the research?

Efforts will be made to limit the use and disclosure of your personal information, including research study data, to people who have a need to review this information. However, we cannot promise complete secrecy. Organizations that may inspect and copy your information include the Institutional Review Board at Arizona State University and other representatives of this organization. All data and blood samples collected during this study will be identified only by a number assigned to you, and will be stored in a secure setting in the ABC laboratory located on the ASU downtown campus in Phoenix.

What else do I need to know?

If you agree to participate in the study, written consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury. All test foods will be provided. To help offset costs associated with travel and time, you will receive a $15 Target gift-card at visit 3, and a $15 Target gift-card at the final visit (visit 5).

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, talk to the research team at Arizona State University. You may email or call the investigators [Carol.Johnston@asu.edu (602) 827-2265 or Karen.Sweazea@asu.edu (480) 965-6025] to express any concerns.

This research has been reviewed and approved by the Bioscience Institutional Review Board at ASU. You may talk to them at (480) 965-6788 or research.integrity@asu.edu if:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You want to talk to someone besides the research team.
- You have questions about your rights as a research participant.
- You want to get information or provide input about this research.

Your signature documents your permission to take part in this research.

Signature of participant	Date
Printed name of participant	
Signature of person obtaining consent	Date

ASU IRB IRB # STUDY00004354 | Approval Period 5/17/2016 – 5/16/2017

APPENDIX C

RECRUITMENT FLYER

ARE YOU INTERESTED IN NATURAL STRATEGIES THAT TARGET BLOOD PRESSURE?

ASU is starting a research study to examine the effects of two types of vegetables on blood pressure.

THE NUTRITION PROGRAM AT ASU IS RECRUITING HEALTHY, POST-MENOPAUSAL WOMEN (40-70 YEARS OLD) TO EXAMINE THE EFFECTS OF TWO TYPES OF VEGETABLES ON BLOOD PRESSURE.

Participation will include:

- Traveling to the ASU Downtown Phoenix campus on 5 occasions over an 8-week period (visit 1 will last about 30 minutes, and visits 2-5 will last about 1 hour each.)
- Consuming a vegetable salad or a vegetable medley (canned vegetables), twice per day, during each of the two 10-day intervention phases of the study. The vegetables include celery, spinach, lettuces, green beans, sweet peas, and corn.
- Obtaining blood pressure, blood samples, and cardiovascular function measurements.

You will receive **$30** in Target gift cards.

INTERESTED? Please visit our recruitment site:

https://www.surveymonkey.com/r/VeggiesToLowerBP

APPENDIX D

PRE-SCREENING ONLINE QUESTIONNAIRE

A Comparison of a High-Nitrate versus a Low-Nitrate Diet on Cardiovascular Health in Post-Menopausal Women

Thank you for choosing to participate in this survey!

ASU Nutrition Professors Karen Sweazea and Carol Johnston and their graduate student, Selicia Mayra, are inviting you to participate in this screening process, which will consist of questions specific to your health, demographics, and scheduling availability. You have the right to skip any question(s) you are uncomfortable answering, or to stop this survey at any time.

Your participation in this survey and the research study is completely voluntary, and you may choose to withdraw from the study at any time without consequence. Your responses to this survey, and participation in this research study will be strictly confidential.

If you meet the inclusion criteria for the study, you will be contacted to schedule an in-person appointment at Arizona State University (Downtown Phoenix campus). This initial appointment should last approximately 30 minutes.

For questions concerning this research study, please contact the research team at smayra@asu.edu or (480) 326-4591. Should you have questions about your rights as a participant in this research study, or if you feel you have been placed at risk, please contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance at (480) 965-6788.

By completing the survey below, you are agreeing to be screened for the (Nitrate-enriched Diet to Promote Cardiovascular Health) study, and to be contacted by investigators (via e-mail) to schedule an in-person appointment, should you qualify for the study.

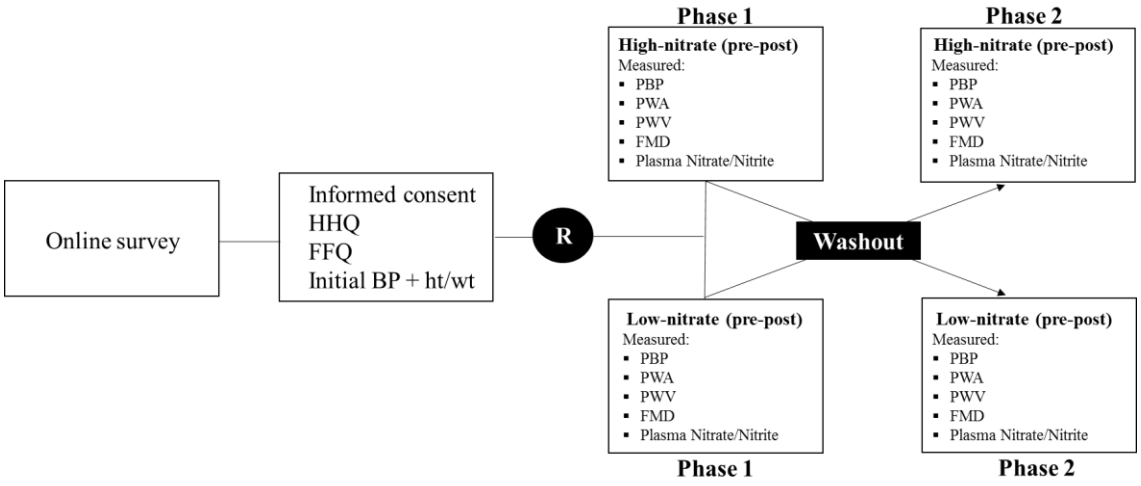
1. Please provide your e-mail address.
2. How old are you (in years)?
3. What is your gender (male or female)?
4. Do you currently have a menstrual period?
5. Have you been told by your doctor that you have hypertension (high blood pressure)?
6. Are you currently on medication for high blood pressure?
7. If you currently use an alcohol-based mouthwash, would you be willing to refrain from further use for the duration of the study?
8. If you currently take aspirin and/or anti-inflammatory medications, would you be willing to refrain from further use for **three** days prior to blood draws?
9. If you currently take a multivitamin/mineral supplement, would you be willing to refrain from further use for **three** days prior to blood draws?
10. If you currently drink alcohol and/or caffeine (e.g., coffee), would you be willing to refrain from further use for **12 hours** prior to blood draws?

11. If you currently smoke, would you be willing to refrain from smoking for **12 hours** prior to blood draws?
12. If you currently exercise, would you be willing to refrain from heavy exercise for **12 hours** prior to blood draws?
13. Would you be willing to refrain from all food and beverages, NOT including water, for **12 hours** prior to blood draws?
14. Are you willing to eat a vegetable salad or a vegetable medley (canned vegetables) twice per day, and also record your food and beverage intake for a 10-day period?
15. Do you have any known food allergies or other food restrictions? If yes, please list _____.
16. Do you currently follow a special diet (e.g., low-fat, DASH, weight loss/weight gain)? If yes, please list _____.
17. In a typical week, how many servings of fruits and vegetables do you eat?
18. Please list examples of fruits and vegetables you eat on a daily or weekly basis.
19. Would you be willing and able to attend 5 in-person visits at the ASU Downtown Phoenix campus? Visit one should last 30 minutes, and visits 2-5 should last 1 hour each.
20. Are you willing to provide fasting blood samples from an arm vein at 4 of the study visits?

Thank you for taking the time to participate in this survey.

APPENDIX E

STUDY PROTOCOL


Phase 1
Phase 2
High-nitrate (pre-post)
Measured:
PBP
PWA
PWV
FMD
Plasma Nitrate/Nitrite
High-nitrate (pre-post)
Measured:
PBP
PWA
PWV
FMD
Plasma Nitrate/Nitrite
Online survey
Informed consent
HHQ
FFQ
Initial BP + ht/wt
R
Washout
Low-nitrate (pre-post)
Measured:
PBP
PWA
PWV
FMD
Plasma Nitrate/Nitrite
Low-nitrate (pre-post)
Measured:
PBP
PWA
PWV
FMD
Plasma Nitrate/Nitrite
Phase 1
Phase 2

APPENDIX F

HEALTH HISTORY QUESTIONNAIRE

Health History Questionnaire

ID#_____

Age:_____

Gender (please circle): Female Male

Smoked cigarettes in the past year?

(please circle): Yes No

To be completed by investigator

Height:_____ cm.

Weight:_____ kg.

1. Are you currently taking any blood pressure medication? Y N

   If yes, what medications and how often?

   _____

   _____

2. Has a doctor ever told you that you have high blood pressure? Y N
3. Do you currently take supplements? (Multivitamins, minerals, herbs, etc.) Y N

   If yes, what supplements and how often?

   _____

   _____

4. Do you have any known food allergies? Y N

   If yes, what foods?

   _____

   _____

5. Are you willing to participate in an 8-week long study, and consume a vegetable salad or a vegetable medley (canned vegetables) twice daily for the intervention phase of the study? Y N
6. Are you willing to provide blood samples from an arm vein on 4 occasions during the study? Y N
7. Are you willing to have your blood pressure taken on 5 occasions during the study? Y N
8. Are you willing to have your blood vessel function assessed on 4 occasions during the study? Y N
9. Are you willing to travel to the ASU Downtown Phoenix campus on 5 separate occasions? Y N
10. Would you be able and willing to refrain from antibacterial mouthwash for the duration of the 8-week study? Y N
11. Would you be able and willing to refrain from aspirin, vitamins/minerals, and anti-inflammatory medication for three days prior to blood draws? Y N

12. Aside from the consuming the vegetable salad or the vegetable medley, would you be willing to maintain your normal diet and physical activity habits during the 8-week study? Y N

13. Do you follow a specific diet? (weight loss/gain, vegetarian, low-fat, etc.) Y N

If yes, please explain.

_____

_____

14. Are you able to follow this pre-visit protocol: refrain from all food and beverages including alcohol, smoking, caffeine, and heaving exercise for 12 hours prior to blood draws? You will be free to drink water during this period. Y N

15. Please circle the total time you spend in each category for an average week.

Light activities such as: slow walking, golf, easy swimming, gardening, etc.

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Moderate activities such as: moderate walking, cycling, swimming, weight lifting, etc.

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Vigorous activities such as: fast walking, jogging, cycling, heavy/intense weight lifting, etc.

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

15. Please describe any other medical conditions or situations that may affect your ability to participate in this research trial (i.e., infections, travel, work deadlines, etc.).

_____

_____

APPENDIX G

FOOD FREQUENCY QUESTIONNAIRE

Subject ID: _____

Please indicate how many times you consumed the following foods IN THE PAST MONTH:

- **Broccoli**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Carrots**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Cauliflower**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Cucumber**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Cabbage**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Dill**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Turnips**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Celery**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Fennel**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Leek**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Parsley**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Lettuce**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Beets** (including juice)
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Spinach**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk
- **Endive**
  ☐ 0 ☐ 1x/mo ☐ 2-3/mo ☐ 1x/wk ☐ 2-3x/wk ☐ 4-5/wk ☐ 6-7x/wk

APPENDIX H

STUDY PROTOCOL CHECKLIST

PID # _____

CHECKLIST

SCREENING (VISIT I)	
Consent + Participant Copy (dated and signed)	
Health History Questionnaire (HHQ)	
Food Frequency Questionnaire (FFQ)	
Future Visits Scheduled 2nd: 3rd: 4th: 5th	
Calendar + Instructions	
Final Report Requested Y / N	
Blood Pressure 1st: _____ 2nd: _____ 3rd: _____ Final: _____ mm Hg	
Height _____ cm	
Weight (Tanita) _____ kg	
Salad Dressing Requested	

BASELINE T1 (VISIT II)		POST INTERVENTION T1 (VISIT III)	
Blood Pressure		Blood Pressure	
PWA/PWV		PWA/PWV	
FMD		FMD	
Blood Draw		Blood Draw	
Weight (Tanita)		Weight (Tanita)	
Supplies (salad + dressing **OR** canned veg)		5-Day Food Diary (x2) returned	
5-Day Food Diary (x2)		Target gift-card I ($15) (get signature)	
Foods to Limit + Ingredients			

BASELINE T2 (VISIT IV)		POST INTERVENTION T2 (VISIT V)	
Blood Pressure		Blood Pressure	
PWA/PWV		PWA/PWV	
FMD		FMD	
Blood Draw		Blood Draw	
Weight (Tanita)		Weight (Tanita)	
Supplies (salad + dressing **OR** canned veg)		5-Day Food Diary (x2) returned	
5-Day Food Diary (x2)		Final Report (if requested)	
		Target gift-card II ($15) (get signature)	