

THE VEGETARIAN DIET AND VASCULAR FUNCTION

by

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ABSTRACT

The health benefits of vegetarian diets are well documented. As compared with their omnivorous counterparts, vegetarians have lower body weight, blood pressure (BP) and cardiovascular disease risk, however, the exact mechanisms of these benefits remain unclear. It is hypothesized that the vegetarian diet may protect the endothelium, which is significant in that vascular endothelial dysfunction is an underlying factor in the pathogenesis of atherosclerosis and high blood pressure (BP). Therefore, the purpose of this study was to determine if individuals who have followed a vegetarian diet for at least five years have improved vascular function when compared to a non-vegetarian diet containing red meat in apparently healthy individuals. Vegetarianism (V) was defined as the absence of meat, poultry, and fish from the diet. Omnivorism (O) was defined as habitual red meat consumption of at least two times per week. Subjects, both men and women, between the ages of 18-45 years were recruited. Assessment of vascular function included brachial artery flow-mediated dilation (FMD), passive leg movement (PLM), pulse wave analysis (PWA), and carotid-to-femoral pulse wave velocity (PWV). A total of 18 subjects, 8 lacto-ovo-vegetarians (age 25.9 ± 2.8 yr; BMI 21.8 ± 0.8 kg/m²) and 10 habitual red meat eaters (age 23.3 ± 1.2 yr; BMI 23.4 ± 0.5 kg/m²), completed the study. Omnivores consumed on average three servings of red meat per week. Significant differences in macronutrient consumption existed between groups with vegetarians consuming more carbohydrate, less fat and protein than omnivores even when normalized

for energy intake. Endothelial function as assessed by FMD (O, $8.91 \pm 3.67\%$; V, $7.65 \pm 3.85\%$) was not statistically different nor was PLM as assessed by peak leg blood flow (O, 681.1 ± 153.7 ; V, 543.0 ± 110.2) between groups. Brachial systolic BP (O, 118 ± 4 ; V, 106 ± 4 ; $p < 0.05$) and central systolic BP (V, 99 ± 3 mmHg; O, 108 ± 3 mmHg; $p < 0.05$) were significantly different. However, no differences in arterial stiffness as assessed by PWA and PWV were seen. In conclusion, following a vegetarian diet for at least five years was associated with improved systolic BP however these differences did not translate to significant differences in vascular function when compared to a non-vegetarian diet including habitual red-meat consumption in healthy individuals.

Chapter 1

INTRODUCTION AND BACKGROUND

1.1 Cardiovascular Disease and Risk Factors

Cardiovascular disease (CVD) is the number one cause of death in the U.S. (Mozaffarian et al., 2016). More than 1 in every 3 adults, an estimated 85.6 million Americans, have at least one type of CVD (Mozaffarian et al., 2016) and by the year 2030, the percentage of the population suffering from CVD is projected to approach 44% (Mozaffarian et al., 2016). CVD is an underlying cause of death for one third of deaths in the U.S., claiming 2,200 lives each day (Mozaffarian et al., 2016). Associated costs of CVD in the U.S. totals an estimated \$316.6 billion annually (Mozaffarian et al., 2016). Risk factors include family history and genetics, age, hypertension, hypercholesterolemia, hyperlipidemia, Diabetes Mellitus, tobacco use, physical inactivity, sub-optimal nutrition, and overweight and obesity (Mozaffarian et al., 2016).

Several epidemiologic studies indicate that red meat intake is associated with increased cardiovascular risk (Ashaye et al., 2011; Bernstein et al., 2010; Chang-Claude et al., 2005; Kontogianni et al., 2007; Micha et al., 2010; Pan et al., 2012; Tuso et al., 2015; Van Dam et al., 2002). Further, a number of these studies demonstrate that this risk is increased proportionately with red meat intake, suggesting a positive linear correlation between red meat consumption and CVD risk (Ashaye et al., 2011; Bernstein et al., 2010; Chang-Claude et al., 2005; Kontogianni et al., 2007; Micha et al., 2010; Pan et al., 2012;

Van Dam et al., 2002). Hence, diets with minimal to no red meat consumption may lessen the risk of CVD.

1.2 The Endothelium

The endothelium is a monolayer of cells lining the blood vessels. It is a large, paracrine organ, playing a vital role in the regulation of vascular tone, cell growth, platelet and leukocyte interactions, and thrombogenicity (Corretti et al., 2002; Libby et al., 2007). A primary function of the endothelium is to maintain vascular homeostasis. Homeostasis is maintained via equilibrium of vasodilators, namely nitric oxide (NO), and vasoconstrictors, including endothelin, thromboxane A₂ (TxA₂) and prostaglandin H₂ (PGH₂) (Libby et al., 2002; Shimokawa, 1999). Recognition of an endothelium-derived relaxing factor, later identified as NO can be credited to the research of Furchgott and Zawadzki (Furchgott & Zawadzki, 1980; Mortensen et al., 2012). The functional importance of the endothelium was first discovered by its effect on vascular tone (Anderson et al., 1995).

Nitric oxide, arguably the most critical substance released by the endothelium, is a potent vasodilator. Stimulation of the endothelium and associated specialized ion channels, such as calcium-activated potassium channels, open in response to shear stress, allowing increased entry of calcium (Cooke et al., 1991). The presence of calcium activates the enzyme endothelial nitric oxide synthase (eNOS), which catalyzes the conversion of L-arginine to L-citrulline thereby producing NO (Pohl et al., 1985; Joannides et al., 1995; Chhabra, 2009). Cofactors involved in this reaction include

nicotinamide adenine dinucleotide (NADPH), tetrahydrobiopterin (BH₄), flavin adenine mono- and dinucleotides (FMN/FAD), and zinc (Félétou, 2011). Vasodilation in response to shear stress or increased blood flow is known as flow-mediated dilation, and is mediated by NO derived from the endothelium (Corretti et al., 2002).

1.2.1 Vascular Function and Cardiovascular Risk

Loss of regulatory function by the endothelium is a risk factor for the development of atherosclerosis, and ultimately, CVD (Widlansky et al., 2003; Chhabra, 2009; Corretti et al., 2002). Endothelial dysfunction describes the partial or total loss of balance between vasoconstrictors and vasodilators, growth promoting and inhibiting factors, and pro and anti-atherogenic factors (Quyyumi, 1998), thereby reducing the production of NO (Lerman & Burnett Jr., 1992; Levine et al., 1995). Since the discovery of NO, it has become evident that changes in its bioavailability play an important role in the development of atherosclerosis, as NO is a key marker of endothelial function and dysfunction (Mudau et al., 2012). Reduced NO formation inhibits the response of the endothelium to vasodilatory stimuli, thereby inhibiting vasodilation (Mudau et al., 2012). Being an early, and reversible initial step in the progression of atherosclerosis and subsequent CVD, early identification of endothelial dysfunction is essential in the prevention of disease progression (Mudau et al., 2012). The assessment of endothelium-dependent vasodilation has emerged as a reliable, non-invasive indicator of endothelial health (Widlansky et al., 2003). Risk factors associated with attenuation of endothelium-dependent vasodilation include smoking, age, hypertension, hyperglycemia, and family

history of atherosclerotic disease. Inflammatory and diet-related factors such as high fat intake have also been identified as risk factors of endothelial dysfunction (Steinberg et al., 1996; Widlansky et al., 2003).

1.2.2 Assessment of Vascular Function

Due to its critical roles in maintaining vascular homeostasis, assessing endothelial function is important, particularly in evaluating cardiovascular risk (Widlansky et al., 2003). Brachial artery FMD is a technique used to measure endothelial-dependent dilation. Measurement of brachial artery FMD in humans correlates well with invasively measured endothelial function of the coronary arteries (Anderson et al., 1995). During assessment of FMD, vasodilation in the brachial artery is stimulated as a result of the release of NO from the endothelium in response to reactive hyperemia and shear stress (Anderson et al., 1995; Corretti et al., 2002). Impaired NO-mediated vascular function is considered an early risk biomarker of CVD (Mozaffarian et al., 2016).

In addition to FMD, PLM is an alternative approach to assess NO-mediated vascular function, and is comparable with FMD (Rossman et al., 2016; Trinity et al., 2012). Passively moving the leg stimulates increases in leg blood flow (LBF) in the femoral artery that are NO-dependent (Trinity et al., 2012). In a study assessing the construct validity of PLM (Rossman et al., 2016), peak change in leg blood flow was significantly correlated with brachial artery and superficial femoral artery FMD, suggesting that PLM provides an analogous indicator of vascular health when compared with FMD (Rossman et al., 2016).

PWA and PWV are considered the standard methods for evaluating arterial stiffness (Kim et al., 2014). Research demonstrates that arterial stiffness measured by PWA and PWV predicts risk of cardiovascular mortality and future cardiovascular events (Vlachopoulos, Aznaouridis, & Stefanadis, 2010; Wang et al., 2010). PWA provides an estimation of the augmentation index (AI), a measure of wave reflection and arterial stiffness (Kim et al., 2014; Laurent et al., 2006; Townsend et al., 2015; Williams et al., 2006). Aortic stiffening leads to loss of normal impedance between the normally pliable aorta and stiff muscular arteries (Townsend et al., 2015). This reduces the amount of wave reflection at the interface between the aorta and proximal branch vessels, increasing pulsatile energy into the periphery, thereby damaging the vessels (Mitchell et al., 2004; Mitchell et al., 2010; Mitchell et al., 2011; Townsend et al., 2015). Arterial stiffness, a major determinant of vascular impedance, is used in combination with the amplitude and timing of wave reflections to determine AI (Townsend et al., 2015). PWV, considered the gold standard for measuring arterial stiffness, assesses the velocity of the pulse wave traveling between the carotid and femoral artery (Townsend et al., 2015). Decreased arterial elasticity and increased stiffness is indicated by a greater pulse wave velocity between these sites (Kim et al., 2014; Laurent et al., 2006). For elastic conduits, the wave velocity is related to the stiffness of the wall, therefore alterations in stiffness modulates the pressure relationships (Townsend et al., 2015).

1.3 Diet and Cardiovascular Risk

The American Heart Association (AHA) describes seven health factors and behaviors, including smoking, having uncontrolled serum glucose, elevated blood pressure and cholesterol, poor diet quality, and being physical inactive, overweight or obese, as being significant contributors to cardiovascular risk (Mozaffarian et al., 2016). According to the U.S. Burden of Disease Collaborators (2013), poor scores related to each of these factors leads to substantial morbidity and mortality in the U.S. Notably, the primary risk factor related to disease burden was found to be suboptimal diet (Mozaffarian et al., 2016; U.S. Burden of Disease Collaborators, 2013). More specifically, insufficient intakes of fruits, vegetables, whole grains, nuts and seeds, in addition to excessive intakes of sodium have been determined to be major contributors to poor diet quality (Mozaffarian et al., 2016).

In addition, the AHA has proposed a series of dietary goals to help Americans increase their diet quality. Goals include meeting recommendations for intakes of fruit, vegetables, and whole grains, and not exceeding recommended limits for intakes of sodium, saturated fat, and processed meat (Mozaffarian et al., 2016). Between 2003 and 2012, the prevalence of American adults meeting these goals (defined as a healthy diet score >80) improved in the U.S., although the percentages remain extremely low (Mozaffarian et al., 2016). Scores on the dietary metric for cardiovascular health showed that the proportion of individuals with poor scores was decreasing steadily (from 50.3% to 41.0%) as healthy diet scores were increasing (from 0.7% to 1.5%) (Mozaffarian et al., 2016).

A report by the U.S. Dietary Guidelines Advisory Committee (U.S. Department of Agriculture, 2015) recently summarized the evidence for the benefits of a healthful dietary pattern on a range of cardiometabolic and other disease outcomes. They concluded that a healthy diet is higher in fruits, vegetables, whole grains, low-fat or nonfat dairy, seafood, legumes, and nuts and lower in red meat, refined grains and added sugars. A report by the U.S. Burden of Disease Collaborators (U.S. Burden of Disease Collaborators, 2013) estimated the impact of all major modifiable risk factors on mortality and morbidity in the United States from 1990 to 2010 and determined that suboptimal diet was the leading cause of both mortality and disability-adjusted life years (DALY) lost, exceeding even tobacco. In 2010, a total of 678,000 deaths of all causes were attributable to suboptimal diet. This was based on fourteen various components of dietary intake. Specifically, when “high processed meat intake” was analyzed as a risk factor, relative risk for morbidity and mortality for CVD was increased by 1.86 fold amongst 25-29 year olds, and 1.79 fold in 30-34 year olds per 50g/day processed meat intake increase.

1.3.1 The Vegetarian Diet and Cardiovascular Risk

The plant-based, vegetarian diet has become recognized as a healthier alternative to a diet laden with meat (Tuso et al., 2015). Traditionally, vegetarianism is interpreted as the avoidance of meat, however, different types of vegetarian diets exist (McEvoy, et al., 2012). The lacto-ovo-vegetarian diet is characterized by the avoidance of meat, poultry, and fish, and allowance of dairy products and eggs. Other subsets of vegetarian groups

include pescatarians, or those who include fish, and vegan, those who abstain from all foods of animal origin, including dairy products and eggs in addition to meat, poultry, and fish (McEvoy et al., 2012; Le & Sabaté, 2014).

Vegetarian diets confer protection against CVD, cardiometabolic risk factors, certain types of cancers and mortality (Le & Sabaté, 2014). Generally, a plant-based diet is low in fat, cholesterol, sodium, and sugar, and as a result, is associated with decreased risk of CVD (Tuso et al., 2015). As compared with omnivores, those who follow a vegetarian diet have lower body mass, blood pressure, LDL-cholesterol, and incidence of cardiovascular events (Lin et al., 2001). This association is well documented in the literature and demonstrated in several large-scale studies nationwide. The most well-known include the Adventist Health Studies amongst Seventh Day Adventists in California (Beeson et al., 1989; Butler et al., 2008; Fraser, 1999; Orlich & Fraser, 2014; Phillips & Kuzma, 1977), The Health Food Shoppers Study (Key et al., 1996), the Oxford Vegetarian Study (Thorogood et al., 1994) in Great Britain, The European Prospective Investigation into Cancer and Nutrition–Oxford (EPIC-Oxford), which was done throughout Europe (Crowe et al., 2013), and the Heidelberg Vegetarian Study (Chang-Claude et al., 1992) in Germany.

Three prospective studies have examined the mortality of vegetarians in Britain, and mortality from ischemic heart disease (IHD) was lower in lacto-ovo-vegetarians in all three studies (Appleby et al., 1999; Key et al., 1996; Key et al., 2003; Thorogood et al., 1994). The EPIC-Oxford cohort, which is the most recent and includes the largest number of vegetarians of any comparable study in the world, reported that vegetarians

had a 32% lower risk of IHD than nonvegetarians, which was adjusted for sex, age, body mass index (BMI), smoking, and the presence of cardiovascular risk factors (Crowe et al., 2013). The reduced mortality seen in the vegetarian population may be related to their serum cholesterol concentrations, which was also reduced in all three cohorts (Key et al., 2003).

Similar to British vegetarians, vegetarians of the Seventh Day Adventist population experience lower rates of CVD and risk factors compared to their non-vegetarian counterparts. In all three Adventist cohorts, (Beeson et al., 1989; Butler et al., 2008; Fraser, 1999; Orlich & Fraser, 2014; Phillips & Kuzma, 1977) lacto-ovo-vegetarians experienced a decreased risk of hypertension, IHD, and all-cause mortality (Le & Sabaté, 2014). In the context of the Adventist studies, vegetarians were defined as lacto-ovo-vegetarian. In the Adventist Health Study, lacto-ovo-vegetarian men had a 37% reduced risk of developing IHD (Fraser, 1999). Most recently in the Adventist Health Study-2 (AHS-2), both male and female lacto-ovo-vegetarians had reduced risk for all-cause mortality and in men for CVD mortality (Orlich & Fraser, 2014). Results from the Adventist cohorts demonstrate an association that is consistent over several years and in an ethnically diverse population (Orlich et al., 2013). Further, vegetarian diets were associated with lower BMI and lower incidence of hypertension, IHD, type 2 diabetes, metabolic syndrome, all-cause mortality, and certain cancers (Le & Sabaté, 2014; Orlich & Fraser, 2014; Phillips & Kuzma, 1977).

A similar decrease in mortality has been reported amongst vegetarians in Germany. The Heidelberg study, which included 1,904 subjects, observed that mortality

from all causes and CVD were lower among those leading a lacto-ovo-vegetarian lifestyle. This was confirmed after 5 and 11 years of follow-up (Chang-Claude et al., 1992). The most significant reduction in mortality was found for CVD. In particular, IHD mortality was reduced to one third that of expected (Chang-Claude et al., 1992).

A collaborative analysis of 5 of these prospective cohorts, including 76,172 participants, concluded that both incidence of and mortality from CVD was lower in individuals following a vegetarian diet (Key et al., 1999). When compared to those who regularly consume meat, mortality from IHD was 34% lower in lacto-ovo-vegetarians (Key et al., 1999), or those who do not consume meat, poultry or fish, but do consume eggs and dairy products. This lower mortality among vegetarians was restricted to those following the diet for greater than 5 years, and was not significantly impacted by confounding factors such as BMI, exercise level, education level or alcohol use (Key et al., 1999). In addition, a meta-analysis of 7 studies including 124,706 participants confirmed that mortality from IHD was significantly lower in the vegetarian population (Huang et al., 2012). Huang et al. (2012) reported that all-cause and circulatory disease mortality was 9% and 16% lower, respectively, in vegetarians when compared to their nonvegetarian counterparts. The significant and consistent associations observed coupled with the absence of confounding factors suggests that the lower mortality from IHD can primarily be attributed to dietary differences amongst vegetarian and nonvegetarian groups (Key et al., 1999).

The vegetarian diet is becoming more widely recognized for its benefits on health. Whether it be due to the avoidance of animal product or the emphasis on plant-based

foods, vegetarian diets are protective against CVD, certain types of cancers and all-cause mortality (Le & Sabaté, 2014). As compared with omnivores, individuals who follow a vegetarian diet have lower body weight, blood pressure, LDL-cholesterol, and incidence of and mortality from cardiovascular events (Lin et al., 2001). Its positive impact on health has been well-documented in several large-scale studies (Beeson et al., 1989; Butler et al., 2008; Chang-Claude et al., 1992; Crowe et al., 2013; Fraser, 1999; Key et al., 1996; Orlich & Fraser, 2014; Phillips & Kuzma, 1977; Thorogood et al., 1994) that are key pioneers in research efforts of the vegetarian population and impact of this diet.

1.3.2 Red Meat Consumption and Cardiovascular Risk

Substantial evidence from epidemiological studies show that meat intake, particularly red meat, is associated with increased risk of CVD (Micha et al., 2010). A number of comprehensive, well-known studies comparing the vegetarian dietary pattern with that of omnivores define high intake as consumption greater than once per week (Beeson et al., 1989; Butler et al., 2008; Crowe et al., 2013; Fraser, 1999; Key et al., 1996; Orlich & Fraser, 2014; Phillips & Kuzma, 1977; Thorogood et al., 1994). In a 21-year follow-up of a German cohort, regular meat intake (defined as consumption >3 times per week) was associated with increased risk of mortality from IHD (Chang-Claude et al., 2005). This risk was significant and trended upward with increased consumption levels of meat (Chang-Claude et al., 2005). An association between consumption of meat and mortality from CVD was observed in the three British vegetarian studies, and was significant in a pooled analysis of five prospective studies, including a follow-up of the

German study (Chang-Claude et al., 2005). These findings are supported by both the Nurses' Health Study and Physician's Health Study, large-scale studies that concluded that higher intakes of red meat were significantly associated with elevated CVD risk in the U.S. (Ashaye et al., 2011; Bernstein et al., 2010). Pan et al. (2012) investigated this association in the Nurse's Health Study in combination with another large prospective cohort, the Health Professionals Follow-up Study (Van Dam et al., 2002). It was determined that a higher intake of red meat, analyzed in quintiles increasing by 1 serving/day, was associated with a significantly increased risk of all-cause and CVD mortality in both men and women. This was for both processed and unprocessed red meat, although greater risk was observed for processed meat (Pan et al., 2012). Participants in the highest quintile of red meat intake experienced a 36% increased risk of CVD when compared with the lowest quintile (Pan et al., 2012). In another study with similar results, food-specific analysis demonstrated that red meat consumption resulted in 52% increased IHD risk, even after controlling for several potential confounding risk factors (Kontogianni et al., 2007). Participants in this study who consumed >8 portions of red meat per month had 4.9 times greater risk of IHD than those who consumed <4 portions of red meat per month (Kontogianni et al., 2007).

1.3.3 Proposed Mechanisms

A plant-based, vegetarian diet avoiding meat, particularly red meat, may exert protective effects against CVD by both increasing factors that are protective to and reducing those that are deleterious to the endothelium (Tuso et al., 2015). Mechanisms

whereby vegetarian diets are protective of the endothelium and result in improved vascular function are thought to include prevention of vascular endothelial cell (VEC) injury, low density lipoprotein (LDL) oxidation, and macrophage activation.

VEC injury may be preventable by consuming foods that are low in sugar, salt, and fat (Tuso et al., 2015). According to the U.S. Burden of Disease Collaborators (2013), the most important dietary risks in the U.S. are diets low in fruits, vegetables, nuts and seeds, and high in sodium, processed meats, and trans fats. This is in contrast to the vegetarian diet which is characterized by high consumption of fruit, vegetables, legumes, nuts, grains and soy protein-food components (McEvoy et al., 2012). Nutrients associated with an increased risk of vascular disease, such as total fat, saturated fat, trans fat and cholesterol are highest in nonvegetarians (Bhupathiraju & Tucker, 2011; Tuso et al., 2015). In contrast, nutrients that are associated with reduced risk of chronic disease, such as fiber and antioxidants, are higher in those consuming a vegetarian diet (McEvoy et al., 2012; Rizzo et al., 2013; Tuso et al., 2015). Differences in intake of specific micronutrients is thought to positively impact those following a vegetarian diet. For example, intake of potassium, which is considered an important nutrient in the prevention of hypertension, is greater in the vegetarian dietary pattern (Rizzo et al., 2013). Nutrient composition and BMI between vegetarians and non-vegetarians is markedly different, despite similar energy intake, suggesting diet composition impacts weight and BMI independent of total energy consumed (Rizzo et al., 2013).

The vegetarian diet pattern may preserve endothelial function via the consumption of antioxidant-containing fruits and vegetables. Increased intake of antioxidants,

including polyphenols, is associated with decreased risk for CVD (Tuso et al., 2015; Mente et al., 2009; Vita, 2005). Polyphenols exert protective effects on VECs by promoting NO production and down-regulating the oxidation of LDL, LDL-induced monocyte adhesion, and transformation to macrophages and foam cells that leads to the development of atherosclerosis (Ginter & Simko, 2012; Tuso et al., 2015; Vita, 2005). Antioxidant rich diets are thought to also prevent atherogenic events such as oxidative stress, inflammation, smooth muscle cell proliferation, and platelet aggregation (Ginter & Simko, 2012; Šebeková et al., 2006; Vita, 2005).

Finally, decreasing consumption of red meat aids in prevention of macrophage activation (Tuso et al., 2015). Studies show a mechanistic association between intestinal microbial metabolism of red meat components, specifically choline and L-carnitine, and CVD (Koeth et al., 2013). This association stems from the production of the pro-atherogenic metabolite, trimethylamine-N-oxide (TMAO) (Koeth et al., 2013). Elevated TMAO levels have been associated with an increased risk of major cardiovascular events (Yang et al., 2011). TMAO causes macrophage activation (Tuso et al., 2015; Wang et al., 2011) resulting in atherosclerosis, and has been identified as a predictive marker of CVD (Tuso et al., 2015; Yang et al., 2011). Additionally, nonvegetarians have been found to produce more TMAO than their vegetarian counterparts who consumed a primarily plant-based diet (Tang et al., 2013). However, research suggests that following a plant-based diet and the development of a plant-based diet microbiota will inhibit the conversion of both choline and L-carnitine to TMAO, even with the occasional consumption of meat (Tuso et al., 2015). Hence, the metabolism of these components to TMAO by the

intestinal microbiome is associated with atherosclerosis in omnivores, but not in vegetarians, further supporting the beneficial effects of a plant-based diet (Tuso et al., 2015).

Many dietary and lifestyle factors, including high intakes of meat and fat, are implicated in the development of CVD (Tuso et al., 2015). Research suggests that lifestyle modification and incorporation of a plant-based diet may play an important role in disease prevention (Tuso et al., 2013; Tuso et al., 2015). Enhancing the ratio of protective to injurious factors via modification of diet is a beneficial approach in CVD prevention and treatment (Tuso et al., 2015).

1.4 Aims and Hypotheses

Several large studies (Beeson et al., 1989; Butler et al., 2008; Chang-Claude et al., 1992; Appleby et al., 1999) provide evidence that those who follow a vegetarian diet have lower blood pressure, body weight, and lower incidence of heart disease and cancer. However, to date, there is little research examining differences in vascular function amongst vegetarians and omnivores. We were therefore interested in investigating whether following a long-term vegetarian diet resulted in better vascular health as compared to otherwise similar omnivores that may explain differences in disease risk and mortality seen in these large studies. The following aim and hypothesis was proposed:

Specific Aim: To determine if vascular function is greater in individuals who have consumed a vegetarian diet for at least five years as compared to those consuming a non-vegetarian diet including habitual red-meat consumption.

Hypothesis: Individuals following a vegetarian diet will have improved endothelial function and reduced arterial stiffening as assessed by brachial artery FMD, PWA, PWV, and PLM.

Chapter 2

METHODS

2.1 Cardiovascular Disease and Risk Factors

Subjects, both men and women, between the ages of 18 and 45 who met the inclusion criteria were invited to participate, including both vegetarians and omnivores. Inclusion criteria for vegetarians included at least 5 years of following the diet with no occasional consumption of meat, poultry, or fish and for omnivores to consume red meat at least 2 times per week. Exclusion criteria for all included a history of heart disease, high blood pressure, diabetes, cancer or kidney disease, use of heart or blood pressure medications, obesity ($\text{BMI} \geq 30 \text{ kg/m}^2$), pregnancy, lactation, being a smoker, or being a highly-trained endurance athlete.

Participants were asked to come to the Health Sciences Complex at the Science, Technology, and Advanced Research (STAR) campus at the University of Delaware for two visits, that included a screening visit and a lab testing visit.

2.2 Visit 1: Informed Consent and Subject Screening

Subjects were asked to come to the Health Sciences Complex at the STAR Campus to read and sign the informed consent form and fill out a medical history questionnaire. Once a participant was found to qualify for the study in visit 1, they were asked to return for visit 2. During the screening, eligible participants were asked to

complete the Global Physical Activity Questionnaire (GPAQ) and the Diet History Questionnaire II (DHQ-II) detailing habitual dietary intake. Female subjects were also asked to fill out a form describing their menstrual cycle and date of their last menses. Height, weight, and body composition were assessed during this visit. Body composition was assessed using the method of bioelectrical impedance via a Tanita scale (TBF-310GS Total Body Composition Analyzer). Resting BP was assessed using the GE DinaMap ProCare 100 Patient Monitor.

2.2.1 Diet Analysis

Subjects completed the Diet History Questionnaire-II (DHQ-II), a food frequency questionnaire, for the purposes of determining vegetarianism or omnivore status. Vegetarianism, in this study, was defined as the absence of meat, poultry, and fish from the diet. The DHQ-II is a web-based questionnaire offered through the National Cancer Institute at the National Institutes of Health. It included 135 food items, questions about portion size, and had a period of recall of 1 year. Subjects followed automated skip patterns based on their responses, and were allowed to navigate within the tool to modify responses at any time. Data quality was ensured as the questionnaire could not be completed with missing or inconsistent responses (National Institutes of Health, 2015). Data collected from the DHQ-II was analyzed using Diet*Calc, DHQ-II associated analysis software. Diet*Calc estimated intakes of both nutrients and food groups. The software consists of three components: the Diet*Calc Analysis, which generates nutrient estimates, the Data Dictionary Editor, which contains information necessary to interpret

data, including file locations and coding information, and the Database Utility, a program that allows importation of nutrient data into the database (National Institutes of Health, 2015).

2.3 Visit 2: Assessment of Vascular Function

Subjects came to the Vascular Physiology Lab at the STAR campus following an overnight fast (6-hour fast for afternoon visits), no caffeine or alcohol consumption for the past 12 hours, and no exercise for the last 24 hours. Weight, body composition, and blood pressure were assessed again. Blood was drawn to measure hemoglobin and hematocrit, cholesterol, and triglycerides. Subjects rested comfortably in a supine position for 20 minutes prior to the vascular function measurements. Assessment of vascular function included PWA to determine augmentation index (AI), carotid-to-femoral PWV, brachial artery FMD, and PLM. Women were tested in the early follicular phase of the menstrual cycle, determined via the Menstrual Cycle Form at visit 1.

2.3.1 Pulse Wave Analysis

Pulse wave analysis, a measure of arterial stiffness, was used to determine wave reflection as assessed by AI. For this test, the subject was in the supine position with one arm parallel to the body, palm facing up. A blood pressure cuff was placed over the brachial artery and pressure was recorded. A central aortic pressure waveform was synthesized from the measured brachial artery pressure waveform using a generalized transfer function (SphygmoCor, AtCor Medical, Sydney, Australia). The amplitude of

central systolic arterial wave reflection was estimated by the AI, which was obtained from the configuration of the generated aortic pressure waveform. In adults of average height, a distinct inflection point (P_i) occurs in systole either before or after peak systolic pressure (P_s) (Salles-Cunha, 1998; Ferreira, 2014). AI is calculated as $(P_s - P_i)/(P_s - P_d)$, where P_s = systolic pressure, P_d = diastolic pressure, and P_i = pressure at the inflection point. Mean arterial pressure (MAP), heart rate (HR), and arterial pressure (AP) were also measured.

2.3.2 Carotid-To-Femoral Pulse Wave Velocity

PWV is a measure of regional aortic stiffness. Carotid-to-femoral pulse wave velocity (PWV) was measured by simultaneously recording ECG and a carotid and femoral pressure wave. The subject was in the supine position. A blood pressure cuff was placed over the femoral artery. A cloth tape measure was used to measure the distances between the carotid artery and sternal notch, and the sternal notch and the femoral artery. A tonometer was placed on the carotid artery, and a carotid and femoral pressure wave was recorded. The distance from the carotid measurement point to the sternal notch was subtracted from the distance from the sternal notch to the femoral measurement point and used as propagation distance. The time delay from the peak of the R wave on the ECG to the upstroke of the carotid pressure wave was subtracted from the time delay from the peak of the R wave on the ECG to the upstroke of the femoral pressure wave. PWV was calculated as propagation distance/time delay.

2.3.3 Brachial Artery Flow-Mediated Dilation

Brachial artery FMD was used to assess endothelium-dependent dilation according to established guidelines (Corretti et al., 2002). Endothelium-dependent dilation is the vasodilatory response to hyperemia and was measured using a 10 MHz linear phased array ultrasound transducer (Terason, uSmart 3300). The subject was in the supine position with one arm extended perpendicular to the body, palm facing up. The brachial artery was imaged above the antecubital fossa in the longitudinal plane (Corretti et al., 2002). After recording baseline images, a cuff placed just below the antecubital crease was inflated to 200 mmHg. The cuff remained inflated for 5 minutes, and then released. Images continued to be recorded through cuff release, and for 2 minutes following deflation. Flow-mediated dilation (FMD) was used as a measure of endothelial dependent function and expressed as a percent change from baseline calculated from vessel diameter data.

2.3.4 Passive Leg Movement

Passive leg movement is a method of assessing NO-mediated vascular function (Trinity et al., 2012). The PLM protocol was performed with the subject in an upright/seated position. It consisted of a 1-minute resting baseline measurement followed by a continuous 2-minute passive leg extension. NO-mediated vasodilation was measured in the femoral artery using an ultrasound system (NextGen LOGIQ e Ultrasound). Continuous PLM was achieved by a member of the research team moving the subject's leg through a 90-degree range of motion at 1 Hertz. The starting position of the leg was in

full extension at the knee (i.e. 180-degree) and the first movement served to passively flex the knee (i.e. move to a 90-degree knee joint angle). Before the start of and throughout the protocol, subjects were encouraged to remain passive and resist the urge to assist with leg movement. To avoid the startle reflex and active resistance to the passive movement, subjects were made aware that passive movement will take place in 1 minute, but to minimize the chance of an anticipatory response, they were not informed of exactly when this movement was to initiate. Measurements of femoral arterial blood velocity and vessel diameter were performed in the passively moved leg distal to the inguinal ligament and proximal to the deep and superficial femoral bifurcation. Vessel diameter was determined at a perpendicular angle along the central axis of the scanned area both prior to and during passive movement. Blood velocity was measured using the same transducers with a frequency of 5 MHz. Arterial diameter was measured, and mean velocity (V_{mean}) (angle corrected, and intensity-weighted area under the curve) was calculated. Blood flow was calculated using arterial diameter and V_{mean} .

2.3.5 Blood Analysis

A venous blood sample was taken to measure hemoglobin (HemoCue Hb 201p model, Lake Forest, California, USA), hematocrit (Clay Adams Brand, Readacrit Centrifuge, BD Diagnostics, Sparks, Maryland, USA), and a lipid profile was obtained. Analysis of the lipid profile was done by LabCorp through the Nurse Managed Primary Care Center at the University of Delaware's STAR campus.

2.4 Statistical Analysis

Variables of interest included percent change in brachial artery FMD, AI calculated from PWA, PWV, and NO-mediated vasodilation from PLM. Unpaired t-tests were performed to assess differences between the two groups for those variables. Descriptive statistics were presented as means $\pm$ standard error of the measure. JMP was used to run the statistical analysis (JMP Pro 13). A *P* value of $P < 0.05$ was considered statistically significant.

Chapter 3

RESULTS

3.1 Subject Characteristics

Eighteen subjects completed this cross-sectional study examining a vegetarian diet compared to a diet containing red meat on vascular function. Of the completed subjects, eight (44%) were lacto-ovo-vegetarians, and ten (56%) were habitual red meat eaters. Amongst vegetarians, number of years following the diet ranged from 6-25 years (14.3 ± 2.5 years). All male subjects fell into the omnivore group and all vegetarians were female. Subjects were mostly White/Caucasian (67%), two were Black/African American (11%), three (17%) were Asian/Asian American, and two (11%) identified with more than one race. Subject characteristics are described in Table 3.1. The differences in height and weight are not unexpected given the sex distribution between the groups. There was a statistically significant difference in vigorous-intensity physical activity between the two groups. Finally, consistent with the literature, brachial systolic BP was different between groups however diastolic BP was not.

Table 3.1 Subject Characteristics

Variable	Omnivores	Vegetarians
Demographic Characteristics		
Age, yr	23.3 ± 1.2	25.9 ± 2.8
Sex (M/F)	7/3	0/8*
Height, cm	179.0 ± 2.6	163.8 ± 2.0*
Weight, kg	75.3 ± 2.4	58.5 ± 2.2*
BMI, kg/m ²	23.4 ± 0.5	21.8 ± 0.8
Physical Activity, hours/week	12 ± 3	8 ± 2
Vigorous-intensity	4 ± 1	2 ± 1*
Moderate-intensity	7 ± 2	5 ± 2
Hemodynamic Measurements		
Heart rate, beats/min	64 ± 4	64 ± 3
Systolic BP, mmHg	118 ± 4	106 ± 4*
Diastolic BP, mmHg	71 ± 3	70 ± 4
MAP, mmHg	84 ± 4	82 ± 3

Values are means ± SE. BMI, body mass index; BP, blood pressure; MAP, mean arterial pressure. *p < 0.05.

3.2 Diet History

A food frequency questionnaire with a one-year period of recall through the National Cancer Institute was used to assess habitual intake. Dietary data are described in Table 3.2. There were significant differences in intake between groups even when normalized to energy intake (Table 3.2) Further, our habitual red meat eaters consumed 3 ± 1 servings/d of red meat, which exceeded the inclusion criteria for this study. When poultry and fish consumption was added to servings of red meat, omnivores consumed on average 8 ± 1 servings/d.

Table 3.2 Diet History

Variable	Omnivores	<i>Omnivore Normalized</i>	Vegetarians	<i>Vegetarian Normalized</i>
Energy, kcal/d	2979 ± 479	-	1484 ± 187*	-
Macronutrients, %				
Total Carbohydrate	36 ± 2	-	54 ± 3	-
Total Protein	18 ± 1	-	13 ± 1*	-
Total Fat	39 ± 1	-	34 ± 2*	-
SFA	13 ± 1	-	9 ± 1*	-
MUFA	15 ± 0	-	14 ± 2	-
PUFA	7 ± 0	-	8 ± 1	-
Macronutrients, g				
Total Carbohydrate	279 ± 53	89 ± 6	193 ± 21	134 ± 8*
Total Protein	132 ± 21	45 ± 2	50 ± 7*	33 ± 1*
Total Fat	128 ± 20	44 ± 1	57 ± 9*	38 ± 3*
SFA	44 ± 8	15 ± 1	15 ± 3*	10 ± 1*
MUFA	48 ± 7	17 ± 1	23 ± 4*	15 ± 2
PUFA	23 ± 3	8 ± 1	13 ± 3*	9 ± 1
Nutrient				
Fiber, g	20 ± 3	7 ± 0	28 ± 4	19 ± 3*
Cholesterol, mg	637 ± 120	232 ± 35	95 ± 34*	67 ± 25*
Na ⁺ , mg	4674 ± 691	1604 ± 48	2254 ± 227*	1628 ± 182
K ⁺ , mg	3995 ± 649	1375 ± 89	3229 ± 447	2293 ± 418*
Mg ⁺ , mg	432 ± 72	149 ± 9	422 ± 61	298 ± 50*
Ca ⁺ , mg	1551 ± 326	506 ± 62	913 ± 132*	632 ± 79
Vitamin B12, mcg	9 ± 2	3 ± 0	2 ± 1*	1 ± 0*
Food Group, servings				
Grains	7 ± 1	2 ± 0	3 ± 1*	2 ± 1
Whole	1 ± 0	0 ± 0	1 ± 0	1 ± 0*
Refined	6 ± 1	2 ± 0	2 ± 0*	2 ± 0
Meat, poultry, & fish	8 ± 1	3 ± 0	0 ± 0*	0 ± 0*
Red meat	3 ± 1	1 ± 0	0 ± 0*	0 ± 0*
Dairy	3 ± 1	1 ± 0	1 ± 0	1 ± 0
Fruit	1 ± 0	0 ± 0	3 ± 1	2 ± 0*
Vegetables	2 ± 0	1 ± 0	3 ± 1	3 ± 1

Values are mean ± SE. Normalized data has been normalized to energy intake per 1,000 kcal. When denoting statistical significance, raw vegetarian data was compared to raw omnivore data, and normalized vegetarian data was compared to normalized omnivore data. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Ca²⁺, calcium; K⁺, potassium; Mg⁺, magnesium; Na⁺, sodium. *p < 0.05.

3.3 Assessment of Vascular Function

3.3.1 Endothelial Function

Endothelial function was examined by performing brachial artery FMD. As shown in Figure 3.1, percent change in FMD was slightly higher in omnivores ($8.91 \pm 3.67\%$) than vegetarians ($7.65 \pm 3.85\%$), however, this was not statistically significant. Baseline and peak diameters as well as diameter change are shown in Table 3.3 There were no differences between the two groups for these diameters.

Table 3.3 Flow-Mediated Dilation

Variable	Omnivores	Vegetarians
Baseline Brachial Artery Diameter, mm	4.3 ± 0.2	3.5 ± 0.1
Peak Brachial Artery Diameter, mm	4.7 ± 0.2	3.8 ± 0.2
Brachial Artery FMD, Δ mm	0.4 ± 0.0	0.3 ± 0.1

Values are mean $\pm$ SE. FMD, flow-mediated dilation.

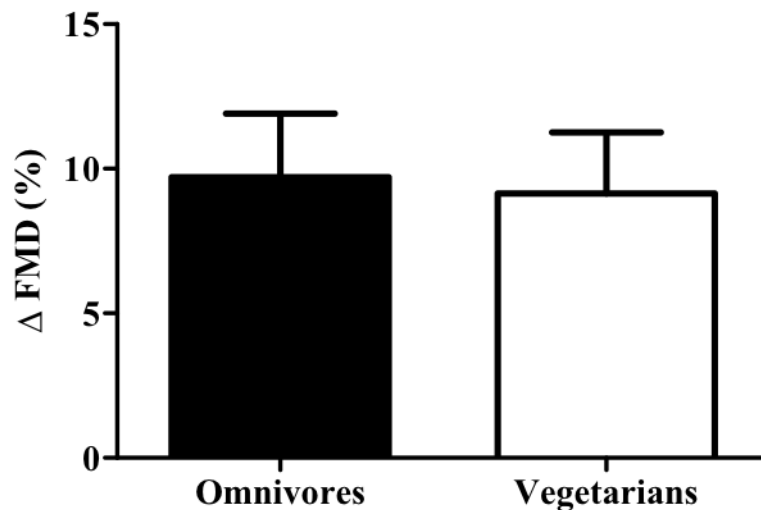


Figure 3.1 Flow-Mediated Dilation

Passive leg movement was used as another approach to assess potential differences in vascular function between vegetarians and omnivores using blood flow in the femoral artery during passive movement of the leg. There were no significant differences in femoral blood flow area under the curve as shown in Table 3.4. Group mean leg blood flow data is shown in Figure 3.2.

Table 3.4 Passive Leg Movement

Variable	Omnivores	Vegetarians
Baseline Femoral Artery Blood Flow, mL/min	268.3 $\pm$ 44.2	187.3 $\pm$ 48.6
Peak Femoral Artery Blood Flow, mL/min	681.1 $\pm$ 153.7	543.0 $\pm$ 110.2
PLM Area Under the Curve, ml	125.6 $\pm$ 45.6	91.1 $\pm$ 32.7

Values are mean $\pm$ SE. *p < 0.05.

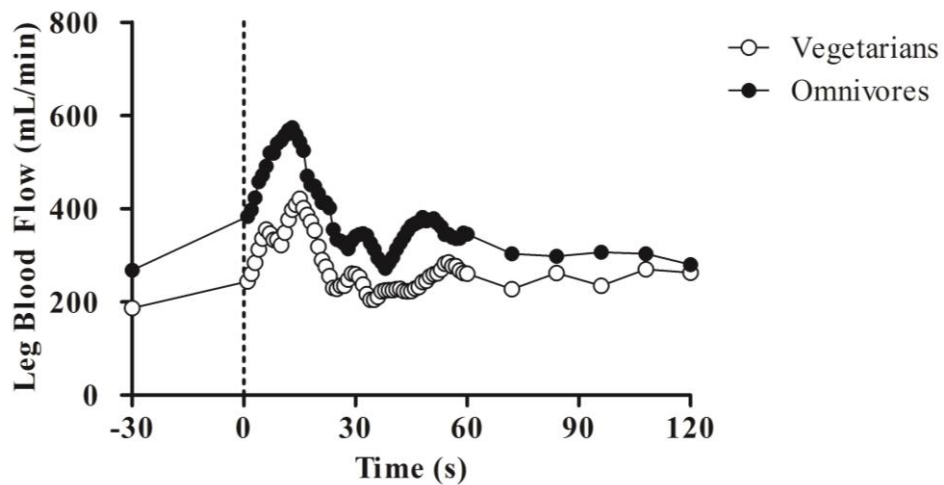


Figure 3.2 Passive Leg Movement One minute of resting data was collected before passive movement, and is represented as a mean baseline flow at time -30. The transition from rest to passive movement occurred at time 0 on the x-axis. Data was collected second-by-second for time 0-60, followed by 12-second averages for time 60-120.

3.3.2 Arterial Stiffness and Wave Reflection

To assess potential differences in stiffness, measures of AI and PWV were performed. There was no significant difference in AI between groups (see Table 3.5). A significant difference was seen in central systolic BP between groups ($p < 0.05$) demonstrating that vegetarians have a lower pressure centrally. However, there were no difference in other central pressures including diastolic BP or MAP. PWV is shown in Figure 3.3. There was no difference between groups.

Table 3.5 Pulse Wave Analysis & Pulse Wave Velocity

Variable	Omnivores	Vegetarians
Pulse Wave Analysis		
Central systolic BP, mmHg	108.1 ± 3.0	$98.8 \pm 2.8^*$
Central diastolic BP, mmHg	74.2 ± 3.6	66.5 ± 4.0
MAP, mmHg	82 ± 3	82 ± 3
AI, %	13.9 ± 3.2	6.2 ± 5.5
Pulse Wave Velocity, m/s	5.6 ± 0.3	5.4 ± 0.4

Values are means $\pm$ SE. AI, augmentation index; BP, blood pressure; MAP, mean arterial pressure. $*p < 0.05$.

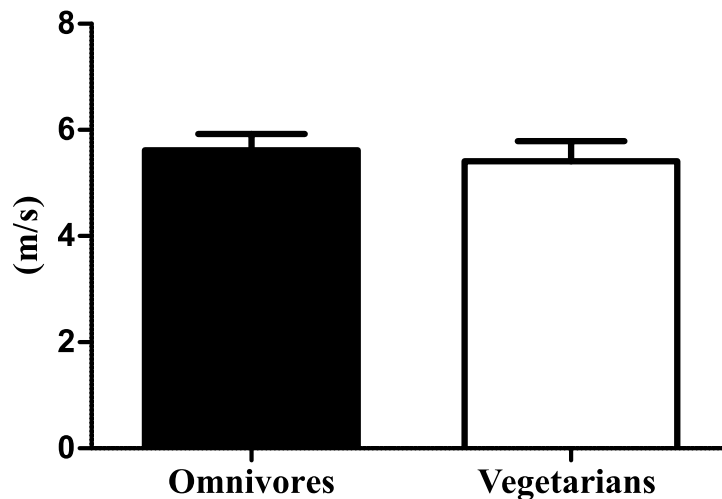


Figure 3.3 Pulse Wave Velocity

3.4 Blood Chemistry/Lipid Panel

Blood was drawn at the screening visit to assess iron status and the lipid panel. There were significant differences in hemoglobin, total cholesterol, and triglycerides between vegetarians and omnivores. Hematocrit, HDL-cholesterol, and LDL-cholesterol were not significantly different between the two groups. Blood chemistry and lipid panel values can be found in Table 3.6.

Table 3.6 Blood Chemistry and Lipid Panel

Variable	Omnivores	Vegetarians
Blood Chemistry		
Hemoglobin, mg/dL	14.1 ± 0.4	12.9 ± 0.5*
Hematocrit, %	43 ± 1	41 ± 1
Lipid Panel		
Total cholesterol, mg/dL	152 ± 6	179 ± 10*
High-density lipoprotein, mg/dL	61.5 ± 3.2	68.8 ± 7.7
Low-density lipoprotein, mg/dL	81.4 ± 7.3	87.4 ± 10.2
Triglycerides, mg/dL	46.5 ± 5.0	116.6 ± 15.8*

Values are means ± SE. *p < 0.05.

Chapter 4

DISCUSSION

The purpose of this study was to determine if vascular function was greater in individuals who have consumed a vegetarian diet for at least five years as compared to those consuming a non-vegetarian diet including habitual red-meat consumption. We hypothesized that endothelial function would be improved and arterial stiffness would be reduced in individuals following a long-term vegetarian diet. Findings from this study indicate no significant difference in vascular function in this small sample of subjects. We did however see a significantly lower brachial and central systolic BP in our vegetarian subjects which is consistent with the purported benefits of a vegetarian diet. This may relate to a reduction in future CVD risk.

4.1 Diet History

As expected, dietary patterns differed substantially between groups. Differences in energy intake can partly be attributed to differences in BMR between sexes (Ferraro et al., 1992), as our vegetarians were all female and less active. Differences can also partly be attributed to diet pattern. Greater consumption of fruits and vegetables, which are less energy dense, was reported in our vegetarian group. Even when normalized to energy intake, there were significant differences in

macronutrient (carbohydrate, protein, and fat) intake. Significant differences also persisted in saturated fat, cholesterol, and vitamin B12 intake, which were higher in omnivores, and dietary fiber, potassium, magnesium, and calcium intake, which were higher in vegetarians. This was not unexpected, as the nutrient-density of vegetarian diets is well-documented in the literature (Le & Sabaté, 2014; Melina, Craig, & Levin, 2016; Tuso et al., 2015). Compared to an omnivorous diet, vegetarian diets contain more whole grains, fruits, and vegetables, and less processed high fat, high sodium, and cholesterol-rich items (Lu et al., 2000; Pan et al., 1993; Tuso et al., 2015), as reflected in our results. Further, our habitual red meat eaters consumed 3 ± 1 servings/d of red meat, which exceeded the inclusion criteria for this study, and is above the definition of high red meat intake in accordance with multiple studies in the field (Beeson et al., 1989; Butler et al., 2008; Crowe et al., 2013; Fraser, 1999; Key et al., 1996; Orlich & Fraser, 2014; Phillips & Kuzma, 1977; Thorogood et al., 1994).

4.2 Blood Pressure

Blood pressure was assessed both at the brachial artery and centrally in our subjects. Both brachial and central systolic BP were notably lower in the vegetarian subjects. The findings of the present study are consistent with those of the Dietary Approaches to Stop Hypertension study (Appel et al., 1997; Sacks et al., 1995), which was based on the observation that components of the vegetarian diet, for example diets rich in fruits and vegetables, was associated with a reduced risk of hypertension and reduced systolic and diastolic BP. In the present study, vegetarians consumed 2 more

servings/d of both fruits and vegetables than omnivores. Further, diets higher in unsaturated fats, vegetable protein, potassium, magnesium, and dietary fiber, and lower in saturated fats are associated with lower BP (Berkow & Barnard, 2005; Elliott et al., 2006; Stamler et al., 1996; Yokoyama, 2014). Specifically, potassium, which is more abundant in vegetarian diets (Rizzo et al., 2013), has been shown in randomized clinical trials to decrease BP (Aburto et al., 2013; Whelton et al., 1997). Indeed, our vegetarian subjects consumed 67% more potassium than our omnivores even after normalizing for energy intake. It is hypothesized that greater potassium intake increases vasodilation and glomerular filtration rate, and decreases renin levels, renal sodium reabsorption, reactive oxygen species production, and platelet aggregation (McDonough & Nguyen, 2012), thereby decreasing BP. A meta-analysis of 7 controlled trials and 32 observational studies of vegetarians by Yokoyama et al. (2014) found that vegetarianism was associated with a lower systolic BP, a difference of -4.8 mm Hg in controlled trials and -6.9 mm Hg in observational studies, compared with omnivorism. The present study yielded similar results; a difference of -12 mm Hg between groups for systolic BP. Whelton et al. (2002) found that a reduction in systolic BP of just 5 mm Hg resulted in a 9% reduction in coronary heart disease mortality. Reductions in systolic BP is beneficial in that high values (above 115 mm Hg) account for almost half of IHD cases (Murray et al., 2003). Hence, our vegetarian group has a reduced risk for cardiovascular disease.

4.3 Endothelial Function

4.3.1 Brachial Artery Flow-Mediated Dilation

The mean brachial artery FMD of both groups was $8.28 \pm 0.92\%$, which is consistent with other studies investigating healthy adults (Jablonski et al., 2009; Pierce et al., 2009). Further, we controlled for variations in the menstrual cycle, testing all females in the early follicular phase, as FMD has been shown to alter throughout the cycle (Williams et al, 2001). Despite differences in systolic BP, brachial artery FMD, an assessment of conduit artery function, did not differ based on diet in this small sample. This is in contrast to other published work.

Using a similar study design that assessed vascular dilatory function of lacto-ovo-vegetarians and omnivores, Lin et al. (2001) found significant differences in FMD between sex and age matched groups. Potential reasons for this discrepancy include a larger sample size ($n=40$), an even distribution of the sexes between groups, and a longer duration following the vegetarian diet (range, 2–21 yrs; mean, 8 ± 5 yrs). Further, this study included relatively older participants (>50 years of age) while our subjects were on average in their 20s. As the degree of vasodilation correlated positively with the number of years on a vegetarian diet, it is possible that our null results were related to our younger sample of vegetarians who have followed a vegetarian diet for less time. These authors also reported that their findings of improved vascular function in vegetarians was dependent on diet alone, and was independent of other known risk factors of atherosclerosis. However, a notable limitation of this study is the lack of diet history or intake data, which is limited to generic Taiwanese nutritional surveys.

A recent meta-analysis (Schwingshackl & Hoffman, 2014) found that in 17 trials including 2,300 subjects, those adhering to a diet high in fruits, vegetables, plant proteins, whole grains, and unsaturated fats, and low in red meat had significantly improved flow-mediated dilatation. It is likely with more subjects, we may see differences in vascular function between vegetarians and omnivores.

In a study by Kajikawa et al. (2016), FMD was significantly decreased with increased serum triglyceride levels, suggesting that elevated serum triglycerides may be independently associated with impaired endothelial function. Although our vegetarians consumed less fat, they did have higher serum triglycerides when compared to our omnivores. This can potentially be explained by a significantly higher intake of carbohydrates in our vegetarian group. It has been shown that high carbohydrate diets induce elevations in fasting serum triglycerides and cholesterol (Abbasi et al., 2000; Coulston et al., 1983), and that carbohydrate intake was positively associated with serum lipid concentrations (Lofgren et al., 2005).

Finally, differences in vitamin B12 intake may also impact vascular function. Vegetarians have been shown to have an increased prevalence of vitamin B-12 deficiency (Elmadfa & Singer, 2009; Pawlak et al., 2014). Kwok et al., (2012) demonstrated that individuals with subnormal vitamin B12 status have impaired endothelial function and increased carotid intima-media thickness. In the present study, our vegetarian group did have a significantly lower intake of vitamin B12, a vitamin that is naturally found in animal products. Although we did not include serum B12 in our assessment, none of our vegetarian subjects reported taking vitamin B12 supplements, and intake was

significantly lower than omnivores. Kwok et al. (2012) recruited 50 vegetarians of at least 6 years, and treated them with either Vitamin B12 (500µg/day) or placebo for 12 weeks with a 10-week washout period before crossover. Vitamin B12 supplementation significantly improved brachial FMD ($6.3 \pm 1.8\%$ to $6.9 \pm 1.9\%$) with further improvement to $7.4 \pm 1.7\%$ ($p < 0.0001$) after 24 weeks of open label vitamin B12. These results demonstrate that vitamin B12 supplementation in vegetarians with subnormal vitamin B12 status significantly enhances arterial endothelial function (Kwok et al., 2012), and suggest that our lack of significant improvements in endothelial function in vegetarians may be related to vitamin B12 status.

Overall, differences in habitual intake between our two groups was substantial. Vegetarians consumed significantly more dietary fiber, potassium, magnesium, and significantly less total fat, saturated fat, cholesterol, and vitamin B12 than omnivores. Vegetarians also consumed more whole grains, fruits, and vegetables. Although our much smaller sample did not demonstrate an improved endothelial function, other studies show adherence to a diet rich in fruits, vegetables, whole grains and low in red meat, as our vegetarian group is, is associated with significantly improved endothelial function (Schwingshackl & Hoffman, 2014). Although our vegetarians consumed less fat, they did have higher serum triglycerides when compared to our omnivores, which some studies suggest may be inversely related with endothelial function (Kajikawa et al., 2016). In addition, low intakes of vitamin B12, which is associated with impaired endothelial function (Kwok et al., 2012), amongst vegetarians may contribute to our null results. Lastly, vasodilation has been found to be directly, positively correlated with the number

of years of vegetarianism in other work (Lin et al., 2001), suggesting that endothelial function in our vegetarian group may show improvements over time.

4.3.2 Passive Leg Movement

Peak femoral artery blood flow (O, 681.1 ± 153.7 mL/min; V, 543.0 ± 110.2 mL/min) and area under the curve (O, 125.6 ± 45.6 ; V, 91.1 ± 32.7) during PLM, was somewhat consistent with that of other studies in healthy adults (Groot, et al., 2015; McDaniel et al., 2010; Trinity et al., 2012; Rossman et al., 2016). Similar to previous studies (McDaniel et al., 2010; Trinity et al., 2012; Rossman et al., 2016; Venturelli et al., 2012), leg blood flow (LBF) increased at the onset of passive movement (12 ± 1 sec), and remained partially elevated above baseline for the duration of movement. As previously mentioned, our vegetarians were entirely female, and the majority of omnivores were male. Similar to findings by Ives et al. (2013), LBF was not significantly different between sexes. Further, we did control for the menstrual cycle when making our LBF measurements although there are currently no studies demonstrating differences in PLM throughout the menstrual cycle.

4.4 Arterial Stiffness

Arterial stiffness as assessed by PWV and wave reflection as assessed by AI did not significantly differ between groups. Our values for PWV (V, 5.41 ± 0.4 m/s; O, 5.62 ± 0.3 m/s) were consistent with other studies in healthy subjects (Edwards, Roy & Prasad, 2008; Kim et al., 2014). Our values for AI (V, $6.17 \pm 15.57\%$; O, $13.93 \pm 9.99\%$)

were also consistent with published work (Kim et al., 2014; Hayward & Kelly, 1997; Smulyan et al., 1998). Kim et al. (2014) determined that differences in markers of arterial stiffness between males and females exist, demonstrating this in a study of Korean individuals who ranged from 17-87 years. Significant differences were found in both AI and PWV based on both sex and age. They reported that PWV was significantly higher in males than in females (7.78 ± 1.16 vs. 7.64 ± 1.15 m/s, $p = 0.015$), reflecting decreased arterial elasticity and increased aortic stiffness (Kim et al., 2014), which is agreeable to our results. In addition to age, PWV was positively associated with systolic BP (Kim et al., 2014). In our study, the omnivore group had a higher PWV and also a significantly higher systolic BP however this correlation was not significant with our small sample. Further, differences in AI between males and females exist due to variability in height (McGrath et al., 2001; Smulyan et al., 1998). Body height is an inverse determinant of augmentation due to earlier wave reflection (Hayward & Kelly, 1997). Hayward & Kelly (1997) found a significant negative correlation between height and AI that showed a higher AI in women due to their shorter stature. Although this did not seem to be demonstrated in our sample, we did have a small sample size. Likely with a larger sample size, the shorter average stature of females than of males and correspondingly shorter arterial trees, would result in an earlier wave reflection and higher AI in female subjects (McGrath et al., 2001; Smulyan et al., 1998).

4.5 Anthropometric and Biochemical Parameters

Our vegetarian and omnivore groups were well matched for age and BMI.

Omnivores did participate in significantly more vigorous-intensity physical activity, which may have produced cardioprotective effects (Swain & Franklin, 2006). The differences in height and weight between the two groups can likely be attributed to differences in the sex make-up of the respective groups.

Hematological analysis in our subjects revealed significantly higher serum hemoglobin levels in omnivores compared to vegetarians, which is likely due to the vegetarian group being entirely female, and the omnivore group being predominantly male, as females generally have lower hemoglobin levels (Murphy, 2014). Surprisingly, total cholesterol and triglycerides were higher in the vegetarian group, despite lower intakes of foods containing these nutrients in our vegetarian group. Total cholesterol values may be attributed to a slightly higher HDL- and LDL-cholesterol values amongst vegetarians that was still within the normal range. Further, one vegetarian subject revealed that her cholesterol may be elevated due to family history. Triglyceride elevation has been associated with oral contraceptive use in women (Castelli, 1986). Serum triglycerides may have been elevated in our vegetarian group due to use of oral contraceptives in half (50%) of our female, vegetarian subjects. Studies report increases in triglycerides, total cholesterol, and LDL-C in females using hormonal contraceptives (Dilshad et al., 2016; Mohammad et al., 2013). Also, estrogen can influence the lipid profile. Hormonal contraceptives, which contain estrogen, increase triglyceride levels by altering the genomic pathway, in which alterations in estrogen affect hepatic apolipoprotein regulation (Jones et al., 2002; Mendelsohn & Karas, 1999; Sitruk-Ware et al., 2007).

4.6 Limitations

There are several limitations in this study. First, our population is small and we are currently underpowered to fully support our hypothesis. Also, this study included a total of 7 males, all omnivores, and 11 females, the vast majority of which were vegetarians. Participants were recruited through a variety of sources and an effort was made to target campus clubs as well as local grocery stores, farmer's markets and restaurants that catered to a vegetarian population. Despite our efforts, we were unable to recruit any male vegetarians. Further, our vegetarian group had a significantly lower intake of vitamin B-12 than their omnivorous counterparts suggesting that may have suboptimal status, however, we did not measure serum B12 levels. Therefore, the results of this study may not be applicable to the vegetarian population at this point in time, until we are better powered and have an equal distribution of the sexes between groups.

4.7 Conclusions

In conclusion, following a vegetarian diet for at least five years was not associated with significant improvements in vascular function when compared with a non-vegetarian diet including habitual red-meat consumption in healthy individuals. However, vegetarians did exhibit a significantly lower brachial and central systolic BP. A larger, more diverse sample of subjects should be explored in future studies. Further research is warranted assessing a larger sample size with equal sex distribution between groups.

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Appendix A

INSTITUTIONAL REVIEW BOARD APPROVAL LETTER



RESEARCH OFFICE

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

DATE: December 23, 2015

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

STUDY TITLE: [841654-1] Vegetarian Vascular Function Study

SUBMISSION TYPE: New Project

ACTION: APPROVED
APPROVAL DATE: December 23, 2015
EXPIRATION DATE: December 15, 2016
REVIEW TYPE: Full Committee Review

Thank you for your submission of New Project materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office.

Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

Appendix B

MEDICAL HISTORY FORM

MEDICAL HISTORY AND GENERAL INFORMATION FORM

Name: _____ Date: _____
Address: _____
City: _____ State: _____ ZIP: _____
Phone: _____ Age: _____
Emergency Contact: _____ Phone: _____

Do you have or have you had:

	Yes	No
Diabetes Mellitus:	☐	☐
Kidney problems:	☐	☐
High blood pressure:	☐	☐
Heart disease:	☐	☐
Stroke:	☐	☐
Irregular heart rhythm:	☐	☐
Other heart problems (specify):	☐	☐
Peripheral Vascular Disease:	☐	☐
Asthma:	☐	☐
Other lung problems (specify):	☐	☐
Any other significant or chronic problems (specify):	☐	☐

If you answered yes to the last question, what significant or chronic problem do you have?

Do you ever have pain in your chest? ☐ Yes ☐ No

Do you ever have pain in your chest while exercising? ☐ Yes ☐ No

Do you currently smoke? ☐ Yes ☐ No

Appendix C

GLOBAL PHYSICAL ACTIVITY QUESTIONNAIRE

Global Physical Activity Questionnaire (GPAQ)



WHO STEPwise approach to NCD risk factor surveillance

Surveillance and Population-Based Prevention
Prevention of Noncommunicable Diseases Department
World Health Organization
20 Avenue Appia, 1211 Geneva 27, Switzerland
For further information: www.who.int/chp/steps

GPAQ

Physical Activity		
Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person. Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. <i>[Insert other examples if needed]</i>. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity		
Questions	Response	Code
Activity at work		
1	Does your work involve vigorous-intensity activity that causes large increases in breathing or heart heavy loads, digging or construction work] for at least 10 minutes continuously? <i>[INSERT EXAMPLES] (USE SHOWCARD)</i> Yes 1 No 2 <i>If No, go to P 4</i>	P1
2	In a typical week, on how many days do you do vigorous- intensity activities as part of your Number of days	P2
3	How much time do you spend doing vigorous-intensity activities at work on a typical day? Hours : minutes hrs mins	P3 (a-b)
4	Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking <i>[or carrying light loads]</i> for at least 10 minutes continuously? Yes 1 No 2 <i>If No, go to P 7</i>	P4
5	In a typical week, on how many days do you do moderate- intensity activities as part of your Number of days	P5
6	How much time do you spend doing moderate-intensity activities at work on a typical day? Hours : minutes hrs mins	P6 (a-b)
Travel to and from places		
The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship. <i>[insert other examples if needed]</i>		
7	Do you walk or use a bicycle <i>(pedal cycle)</i> for at least 10 minutes continuously to get to and Yes 1 No 2 <i>If No, go to P 10</i>	P7
8	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get Number of days	P8
9	How much time do you spend walking or bicycling for travel on a typical day? Hours : minutes hrs mins	P9 (a-b)
Recreational activities		
The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure), <i>[insert relevant terms]</i>.		
10	Do you do any vigorous-intensity sports, fitness or recreational <i>(leisure)</i> activities that cause large heart rate like <i>[running or football]</i> for at least 10 minutes continuously? <i>[INSERT EXAMPLES] (USE SHOWCARD)</i> Yes 1 No 2 <i>If No, go to P 13</i>	P10
11	In a typical week, on how many days do you do vigorous- intensity sports, fitness or Number of days	P11
12	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day? Hours : minutes hrs mins	P12 (a-b)

GPAQ, Continued

Physical Activity (recreational activities) contd.		
Questions	Response	Code
13 Do you do any moderate-intensity sports, fitness or recreational (<i>leisure</i>) activities that causes a small increase in breathing or heart rate such as brisk walking, (<i>cycling, swimming, volleyball</i>) for at least 10 minutes continuously? <i>[INSERT EXAMPLES] (USE SHOWCARD)</i>	Yes 1 No 2 If No, go to P16	P13
14 In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (<i>leisure</i>) activities?	Number of days	P14
15 How much time do you spend doing moderate-intensity sports, fitness or recreational (<i>leisure</i>) activities on a typical day?	Hours : minutes : hrs mins	P15 (a-b)
Sedentary behaviour		
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television], but do not include time spent sleeping. <i>[INSERT EXAMPLES] (USE SHOWCARD)</i>		
16 How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes : hrs min s	P16 (a-b)

Appendix D
MENSTRUAL CYCLE QUESTIONNAIRE

Subject ID# _____

Menstrual Cycle Questionnaire

Are your periods regular? Yes/No

If yes, what is the usual number of days between periods (from the beginning of one period to the beginning of the next)? _____

If no (irregular periods); periods start every: _____ to _____ days

If irregular, how long have cycles been irregular? _____

Are you using any birth control now (pills, patch, IUD)? _____

What is the date of your last period? _____